

鹿児島大学歯学部紀要

Annals of Kagoshima University Dental School

Volume 27

2007

— 目 次 —

Effects of neuroactive steroid hormones on learning and memory Takashige Nishikawa and Tomoaki Sato ...	1
麻酔薬による一酸化窒素 (NO) を介した食道平滑筋弛緩反応の抑制機序 糀 谷 淳 ...	15
鹿児島大学歯学部発表論文 [2005年 SCI (または JCR) リスト雑誌で公表された業績]	29

鹿 歯 紀

Ann. Kagoshima Dent.

鹿児島大学歯学部紀要投稿規定

1. 本誌は歯科医学の研究や教育に関して特定のテーマに基づき、総説あるいは啓蒙的・解説的な論文を主体に掲載する。本学部の教員は下記の規定に従い、誰でも投稿することが出来る。投稿論文の採否は、編集委員会が決定する。
2. 本誌は年1回発行する。
3. 論文の掲載は受理の順とする。ただし編集委員会より特に依頼した原稿については、別に委員会が定める。
4. 掲載料は無料とし、別刷50部を贈呈する。
5. 和文原稿はA4版またはB5版400字詰め原稿用紙を用いて書き、英文原稿はA4版用紙に10ピッチ、ダブルスペースでタイプする。別にコピー部をつける。原稿をワープロで作成した場合は、フロッピーディスクをつける。
6. 表紙（原稿第一枚目）には、1)表題、2)著者名、3)所属、4)欄外見出し(和文25字以内)、5)図表の数、6)原稿の枚数、7)別刷請求部数(朱書)、8)編集者への希望などを書く。
7. 英文抄録(Abstract)をつけ、その表紙には、1) タイトル、2) 著者名、3) 所属、4) Key words (5 words 以内)、5)抄録本文はA4版タイプ用紙で250語以内とする。
8. 和文中の外国文字はタイプとし、和綴りにするときには片かなとする。イタリック指定をしたいところは、アンダーラインを引きその下にイタリックと書く。動物名などは原則として片かなを用いる。単位及び単位記号は、国際単位系による。
9. 本文の欄外に赤字で図表を挿入すべき位置を指定する。
10. 項目分は、1, 2さらに A, Bさらに 1, 2さらに a, bとように分ける。
11. 文献表の作り方
 - 1) 本文中に文献を引用するときは文中の該当する箇所または著者名の右肩の引用の順に従って、番号を付ける。3人以上連名の場合は、“ら”または“et al.”を用いる。
例1：前田ら³⁾によれば.....
例2：Hodgkin & Huxley¹⁾によれば.....
 - 2) 末尾文献表は引用の順に整理し、本文中の番号と照合する。著者名は、et al.と略さず全員を掲げる。
 - 3) 雑誌は著者：表題、雑誌名、巻、頁(始-終)、西暦年号の順に記す。
例1：3) 前田敏宏, 渡辺 武, 水野 介, 大友信也：B型肝炎ウイルスに対するモノクロナール抗体. 細胞工学, 1, 39-42, 1982
例2：1) Hodgkin, A. L. & Huxley, A. F.: The components of membrane conductance in the giant axon of *Loligo*. J. Physiol. (Lond.), 116, 473-496, 1952
 - 4) 単行本は著者名、書名、版数、編集名、章名、引用頁、発行所、その所在地の順に記す。論文集などの場合は雑誌に準じるが、著者名：章名、書名、版数、編集名、引用頁、発行所、所在地、西暦年号の順に記す。
例1：金子章道：視覚；感覚と神経系(岩波講座現代生物化学8), 初版, 伊藤正男編, 38-57, 岩波書店, 東京, 1974
例2：McElligott, J. G.: Chap 13, Long-term spontaneous activity of individual cerebellar neurons in the awake and unrestrained cat., In; Brain Unit Activity during Behavior, 1st ed., M. I. Phillips, Ed., 197-223, Charles C. Thomas, Spring-field, 1973
 - 5) 孫引きの場合は原典とそれを引用した文献及びその引用頁を明らかにし、“より引用”と明記する。
 - 6) 雑誌名の省略名は雑誌により決めてあるものについてはそれに従い、決めてないものについては日本自然科学雑誌総覧(1969, 日本医学図書館協会編, 学術出版会)またはIndex Medicusによる。これらにないものについては、国際標準化機構の取り決め ISO R4 (ドクメンテーションハンドブック, 1967, 文部省, 大学学術局編, 東京電気大学出版局, 39 - 42頁参照)に従う。
12. その他
集会などの内容紹介、海外だより、ニュース、討論、意見、書評、随筆など歯科医学または歯科医学者に関係あるあらゆる投稿を歓迎する。全て図表、写真などを含めて400字詰原稿用紙5枚以内にまとめる。但し、採否は編集委員会が決定する。
13. 本紀要に掲載された論文、抄録、記事等の著作権は、鹿児島大学歯学部へ帰属する。

編集委員

杉原 一正 仙波 伊知郎
原田 秀逸 山崎 要一
(50音順)

Effects of neuroactive steroid hormones on learning and memory

Takashige Nishikawa and Tomoaki Sato

Department of Applied Pharmacology,
Kagoshima University Graduate School of Medical and Dental Sciences,
Sakuragaoka, Kagoshima 890-8544, Japan

Abstract

Here we describe effects of neuroactive steroids, estrogen and progesterone, on cognitive functions. These neuroactive steroids are synthesized in the central and peripheral nervous system including other tissues. They are involved in the regulation of learning and memory, or mood formation in premenstrual syndrome, and these are related to hormone replacement therapy in addition to postnatal and major depression, anxiety disorders, and Alzheimer's disease. Estrogen and progesterone have their individual receptors. The action of estrogen and progesterone can be direct genomic, indirect genomic, or non-genomic, also influencing several neurotransmitter systems. Estrogen has been related to improved learning and memory, or mood formation. On the other hand, progesterone may have negative effects. In this chapter we introduce following our three experimental results. 1) Effects of ovariectomy and calcium deficiency on learning and memory of eight-arm radial maze. Here we suggested that OVX or low-calcium diet might impair learning and memory and that the combination of these factors impaired more markedly, and these also implied the possibility that a woman in menopause or post-menopause might suffer impairment of learning and/or memory when intakes low-calcium diet¹⁾. 2) Effects of estradiol and progesterone on radial maze performance in rats fed a low-calcium diet. Here we suggested the possibility that treatment with estradiol under low-calcium conditions could not improve impaired learning and memory when progesterone was applied simultaneously, and that the intake of adequate calcium might be necessary and effective for patients with learning and memory hypofunction receiving hormone replacement therapy²⁾. 3) Effects of steroid hormones on (Na⁺, K⁺)-ATPase activity inhibition-induced amnesia on the step-through passive avoidance task. Here we indicated that estradiol and testosterone ameliorated the amnesia induced by inhibition of (Na⁺, K⁺)-ATPase activity, and that the protective effect of estradiol might be caused by a non-genomic, rather than a genomic effect or a radical scavenging action. Additionally, the ameliorative effect of testosterone did not appear to involve free radical scavenging, but its aromatization to estrogen could contribute to the non-genomic action of estradiol³⁾.

Key words: learning and memory, Estradiol, Progesterone, Neuroactive steroids, calcium deficiency, Morris water maze, passive avoidance

Neuroactive steroids

Neuroactive steroids, estradiol and progesterone, are synthesized in the central and peripheral nervous system including neurons and astrocytes^{4,5}. The precursor, cholesterol, is supplied or derived from low-density lipoproteins in many cells including the nervous system^{6,7,8}. The cytochrome P450 side-chain cleavage enzyme (P450scc) is related to the conversion of cholesterol to pregnenolone⁹. Pregnenolone is oxidized to progesterone by the 3 β -hydroxysteroid dehydrogenase/isomerase. Estradiol is converted from testosterone in the peripheral or the brain by aromatase P450. This enzyme is localized in hippocampal neurons-pyramidal cells of the CA1-CA3 regions in addition to the granule cells of the dental gyrus¹⁰. The central nervous system is able to take up the steroids from the blood. These indicate that the brain concentrations are related to the peripheral production in the endocrine organs. Estradiol and progesterone are the major human female sex hormones. In the adult women, the main sources of estradiol are the granulosa cells of the developing follicle and the corpus luteum¹¹. The adrenals can also produce androstenedione, which is converted to estrone and estradiol, or to testosterone as the brain can produce and convert to these steroids.

Progesterone is mainly synthesized in granulosa cells of the corpus luteum as well as in the placenta and the adrenals¹¹. Following synthesis, most of the estradiol and progesterone are bound to plasma proteins such as globulin, albumin, transcortin though bounding hormones are relatively inactive.

Estrogens are required for the normal female phenotype, female genital function, sexual maturation but also for skeleton maintenance and are protective for the cardiovascular system¹² as well as for the central nervous system. Progesterone is a very important hormone for conception and pregnancy maintenance. Ovarian steroids have important effects on brain function, including learning and memory, regulation of the reproductive neuroendocrine system, or mood formation^{13,14}. As neuroactive steroids are lipophilic and relatively small molecules, they readily cross the blood brain barrier and become available for their actions on the central nervous system. Neuroactive steroid concentrations in plasma and the brain vary throughout the menstrual cycle and decrease in woman in menopause or post-menopause.

Neuroactive steroid receptors

Estrogen receptors (ER; ER α and ER β) and progesterone receptors (PR; PR α and PR β) belong to a super family of transcription factors, the nuclear receptor family^{15,16}. ERs consist of individual domains such as N-terminal domain, DNA-binding domain, hinge ligand binding domain, and C-terminal domain¹⁷. These diffuse into the cell and bind to their own receptors with transformation or activation of the receptors. Activation comes from dissociation of the receptor-heat shock protein complex and formed dimerization. The dimer binds to specific DNA hormone response elements in the promoter region of target genes and initiates transcription, subsequently leading to translation¹¹. ER α and ER β can form both homo- or heterodimers^{18,19} as with PR α and PR β ²⁰. Phosphorylation sites in these receptors have been identified, and DNA binding and transcription are modified by phosphorylation. Receptors are phosphorylated in the absence of ligands and exhibit further phosphorylation on ligand binding²¹. Estrogen receptors also be able to regulate transcription through binding to the AP-1 response element²² and are distributed in many organs such as the uterus, ovaries, lungs, breast, and the central nervous system^{23,24}. In the central nervous system, they are localized in the hippocampus, cortex, amygdala, the septum, and the hypothalamus^{25,26,27,28}.

Progesterone receptors are also distributed in many tissues including the central nervous system^{29,30,31}. The action of neuroactive steroid hormones could be direct genomic, indirect genomic or non-genomic³².

The direct genomic mechanism of estrogen involves the association of the estrogen-ER dimer complex with estrogen response element or with the fos/jun heterodimers bound to activator protein 1. The indirect genomic mechanism involves activation of ERs linked to the second messenger systems such as protein kinase A or C, mitogen-activated protein kinase (MAPK), extracellular signal regulated kinase (ERK), cAMP response element binding protein (CREB) and nuclear factor- κ B. Non-genomic effects are the stabilization of the mitochondrial membranes and reduction of the generation or the scavenging of free oxygen radicals, with a resultant neuroprotective effect^{33,34,35}.

Progesterone also has a direct genomic mechanistic action onto its receptor, coupled to co-activators, CREB

binding protein and an indirect genomic action is through GTP binding protein.

Action of steroid hormones also seems to be through neurotransmitter systems including the cholinergic, serotonergic and GABAergic systems^{36, 37, 38}. Furthermore, estradiol induces NMDA receptor expression in the CA1 region of the hippocampus and NMDA receptor antagonists block estrogen-induced synaptogenesis on dendritic spines^{38, 39}.

Experimental results and their outline on learning and memory

1) Effects of ovariectomy and calcium deficiency on learning and memory of eight-arm radial maze in middle-aged female rats¹⁾

To examine the effect of estrogen deficiency and low-calcium diet on learning and memory, middle-aged female Wistar rats (50 weeks old) were fed either a low-calcium (0.02% Ca) or a normal-calcium (1.25% Ca) diet throughout the experiment. Rats were ovariectomized (OVX) or sham-operated (Sham). These animals were divided into four groups: 1) Sham group with normal-calcium diet [Sham-normal Ca group], 2) OVX group with normal-calcium diet [OVX-normal Ca group], 3) Sham group with low-calcium diet [Sham-low Ca group], 4) OVX group with low-calcium diet [OVX-low Ca group]. Seventy-seven days after the OVX or Sham operation, the learning and memory abilities in the female rats were examined by using a radial maze task according to the method of Olton and Samuelson (regular trials) and using a delay-interposed task following regular trials. During regular trials and delay-interposed tasks, the OVX-low Ca group was inferior to all the other groups in accuracy of choice behavior. Both Sham-normal Ca and Sham-low Ca groups showed more accurate choices than the OVX-low Ca group, but were less accurate than the Sham-normal Ca group. In addition, there was no significant difference in locomotor activity between any of the groups. These results suggest that OVX or low-calcium diet may impair learning and that the combination of these factors impaired more markedly when the rats were tested in the eight-arm radial maze. These results may also imply the possibility that a woman in menopause or post-menopause suffers impairment of learning and/or memory when intakes low-calcium diet.

Above abstract was derived mainly from the following experimental results.

Fig. 1 plots number correct until first mistake (Fig. 1A) and total errors (Fig. 1B) in blocks of six trials. In Fig. 1A, a significant effect for each treatment group is observed ($F(3,24)=11.93$, $P<0.01$), relative to trial block ($F(4,12)=42.59$, $P<0.01$) and interaction between treatment groups in the trial blocks ($F(4,96)=5.95$, $P<0.01$). These measurements indicate that the change in the number correct until the first mistake for each treatment group was different during the regular trials (i.e. each treatment group was different in its ability to resolve our radial maze task) and that these subjects, except for the OVX-low Ca group, showed an increase in the number of correct choices as the trials progressed. Furthermore, we also compared each treatment group by contrast analysis. There were significant difference between the Sham-normal Ca and OVX-normal Ca ($P<0.01$), Sham-normal Ca and OVX-low Ca ($P<0.01$), Sham-low Ca and OVX-low Ca ($P<0.01$), and OVX-normal Ca and OVX-low Ca ($P<0.05$) groups. In addition, a comparison by contrast analysis also indicated a significant interaction of the treatments in the trial blocks between Sham-normal Ca and OVX-normal Ca ($P<0.01$), Sham-normal Ca and Sham-low Ca ($P<0.05$), Sham-normal Ca and OVX-low Ca ($P<0.01$), Sham-low Ca and OVX-low Ca ($P<0.01$), and OVX-normal Ca and OVX-low Ca ($P<0.01$) groups. Fig. 1B demonstrates the significant effect of each treatment group ($F(3,24)=9.96$, $P<0.01$), trial block ($F(4,12)=25.01$, $P<0.01$), and interaction between treatment groups in the trial blocks ($F(4,96)=2.85$, $P<0.01$). The results of this analysis also revealed that the change in the total errors for each treatment group was different and that in the treated subjects, total errors decreased as the trials progressed. The comparisons by contrast analysis indicated significant differences between Sham-normal Ca and Sham-low Ca ($P<0.05$), Sham-normal Ca and OVX-low Ca ($P<0.01$), OVX-normal Ca and OVX-low Ca ($P<0.01$), and Sham-low Ca and OVX-low Ca ($P<0.01$) groups. Moreover, contrast analysis for total errors also revealed significant interactions of the treatments in the trial blocks between Sham-normal Ca and OVX-low Ca ($P<0.01$), OVX-normal Ca and OVX-low Ca ($P<0.01$), and Sham-low Ca and OVX-low Ca ($P<0.01$) groups.

Fig. 2 shows the number correct until the first mistake

(A) and the total errors (B) over the 12 delay-interposed radial maze tasks (delay trials). Each time-delay period is the mean of three consecutive delay trials. For the number correct until the first mistake, there were significant treatment effects ($P < 0.01$) at all interposed delay times: 30 min, $F(3,24) = 11.97$; 1 h, $F(3,24) = 7.33$; 2 h, $F(3,24) = 5.25$; 3 h, $F(3,24) = 9.15$. Post-hoc tests indicated that rats in the OVX-normal Ca group had lower scores than those in the Sham-normal Ca group at 30 min ($P < 0.05$) and 3 h ($P < 0.05$), and that Sham-low Ca-group rats also showed lower scores than the Sham-normal Ca group at 30 min ($P < 0.01$) and 2 h ($P < 0.05$). Furthermore, OVX-low Ca-group rats showed lower scores than all the other groups at each interposed delay time. In short, the post-hoc test indicated that OVX-low Ca-group rats had lower scores than not only the OVX-normal Ca group (30 min, $P < 0.05$; 1 h, $P < 0.01$;

2 h, $P < 0.05$; 3 h, $P < 0.01$) but also Sham-low Ca-group rats (1 h, $P < 0.05$; 3 h, $P < 0.01$). For the total errors, there were significant differences ($P < 0.01$) between the four treatment groups at all interposed delay times: 30 min, $F(3,24) = 6.57$; 1 h, $F(3,24) = 15.07$; 2 h, $F(3,24) = 14.54$; 3 h, $F(3,24) = 5.24$. The post-hoc tests indicated that there was no significant difference between the OVX-normal Ca and Sham-normal Ca groups but a trend of increasing total errors was observed in the OVX-normal Ca group, and that total errors for the Sham-low Ca and OVX-low Ca groups were significantly higher than for the Sham-normal Ca group ($P < 0.01$), but there was no significant difference between the Sham-low Ca and OVX-low Ca groups. In addition, OVX-low Ca-group rats showed a significantly increased value for total errors compared to the OVX-normal Ca-group rats at each interposed delay time (30 min, $P < 0.05$; 1 h, $P < 0.01$;

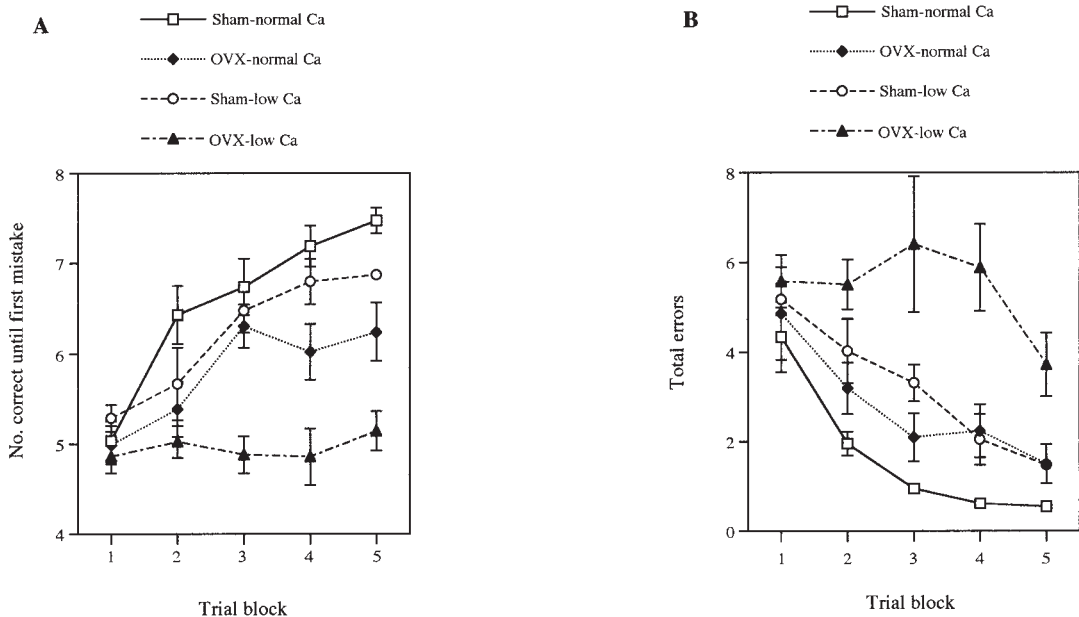


Fig. 1. Changes in the number of correct choices until the first mistake (A) and total number of incorrect choices (B) for maze performance of each treatment group (Sham-normal Ca: normal control diet plus sham-ovariectomy (□), OVX-normal Ca: normal control diet plus ovariectomy (◆), Sham-low Ca: low-calcium diet plus sham-ovariectomy (○), OVX-low Ca: low-calcium diet plus ovariectomy (▲)). Values are the average number of correct choices until the first mistake \pm S.E.M. (A) and the average total number of error choices \pm S.E.M. (B) for seven rats per group. A block is the mean of six consecutive trials. Results are expressed as the mean \pm S.E.M. Choice accuracy data for regular trials were averaged to give blocks of six trials each. The differences in choice accuracy parameters in the regular trials were analyzed by repeated-measures ANOVA, and comparisons of changes between the treatment groups were made by contrast analysis. All the other data among groups were analyzed by one-way analysis of variance (ANOVA) with post-hoc tests (Fisher's partial least square difference (PLSD)). Statistical significance was defined as $P < 0.05$. Data analyses were performed using Super ANOVA 1.11.

2 h, $P<0.01$; 3 h $P<0.01$).

2) Effects of estradiol and progesterone on radial maze performance in middle-aged female rats fed a low-calcium diet²⁾.

To examine the effect of ovarian steroids on learning and under a low-calcium condition, middle-aged female rats were fed either a low-calcium (0.02% Ca) or a normal-calcium (1.25% Ca) diet. All rats were ovariectomized (OVX), and these animals were divided into eight groups: 1) an OVX group with a normal-calcium diet (OVX-normal-Ca group), 2) an OVX group with 17 β -estradiol treatment and a normal-calcium diet (E2 group), 3) an OVX with progesterone treatment and a normal-calcium diet (P4 group), 4) an OVX with 17 β -estradiol and progesterone treatments and a normal-calcium diet (E2+P4 group), 5) an OVX group with a low-calcium diet (OVX-low-Ca group), 6) an OVX group with 17 β -estradiol treatment and a low-calcium diet (LE2 group), 7) an OVX group with progesterone treatment and a low-calcium diet (LP4 group), and 8) an OVX group with 17 β -estradiol and progesterone treatments and a low-calcium diet (LE2+LP4 group). Seventy-seven days after the OVX operation, the learning and memory abilities of the rats were examined by

using an eight-arm radial maze task. E2 and E2+P4 groups learned in fewer trials, and performed better in the radial maze and the working memory task than the other groups under the normal-calcium condition. Rats in the LE2 group learned in fewer trials, and performed better in the maze and working task than the other low-calcium groups, but in combination with progesterone under the low-calcium condition (LE2+LP4 group), the facilitative effect of estradiol in all the tasks was inhibited. Treatment with progesterone alone did not inhibit the learning and memory task performance. These results suggest the possibility that treatment with estradiol under low-calcium conditions cannot improve impaired learning and memory when progesterone is applied simultaneously, and that the intake of adequate calcium may be necessary and effective for patients with learning and hypofunction receiving hormone replacement therapy.

Above abstract was derived mainly from following experimental results²⁾.

Fig. 3 shows the number of trials until the criterion was reached (trials to criterion), the number of correct choices until the first mistake (number correct until first mistake) and the total number of incorrect choices per trial (total errors) in each diet and treatment group. Trials to cri-

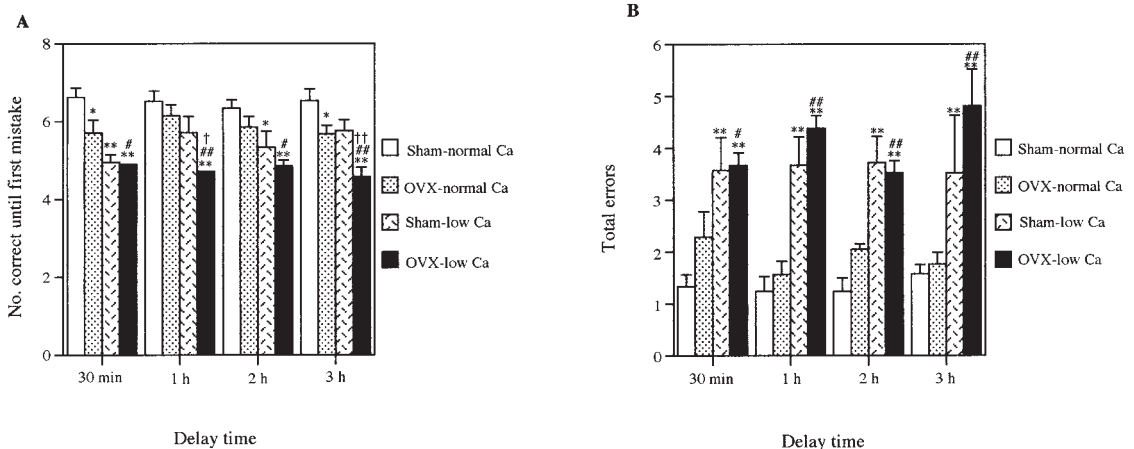


Fig. 2. Effects on performance of introducing each time delay (30 min-3 h) between the fourth and fifth choices. Values are the average number of correct choices until the first mistake \pm S.E.M. (A) and the average total number of incorrect choices \pm S.E.M. (B) for seven rats per group. Each time-delay period is the mean of three consecutive trials. Open bars: normal control diet plus sham-ovariectomy (Sham-normal Ca); dotted bars: normal control diet plus ovariectomy (OVX-normal Ca); hatched bars: low-calcium diet plus sham-ovariectomy (Sham-low Ca); solid bars: low-calcium diet plus ovariectomy. (OVX-low Ca) * $P<0.05$ and ** $P<0.01$ vs. Sham-normal Ca group; # $P<0.05$ and ## $P<0.01$ vs. OVX-normal Ca group; † $P<0.05$ and †† $P<0.01$ vs. Sham-low Ca group.

terion are shown in Fig. 3A and the number correct until the first mistake and the total number of errors are shown in Fig. 3B. The number correct until first mistake and the total errors in Fig. 3B were calculated as the average for the five consecutive trials until the criterion was fulfilled. As for the trial to criterion (Fig. 3A), two-factorial ANOVA revealed significant differences between the normal- and low-calcium diets ($F(1,48)=14.35, P<0.01$) and among the hormonal treatments ($F(3,48)=9.06, P<0.01$) but the interaction between the calcium diet and hormonal treatment was not significant ($F(3,48)=1.66$). In short, low-calcium diet groups had higher scores than the normal-calcium groups (i.e. more trials to criterion). Post hoc tests showed a significant difference between the OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.01$), P4 and E2+P4 ($P<0.05$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) groups. Although statistical significance was not detected, the number of trials until criterion of the OVX-normal-Ca group tended to be lower than that of OVX-low-

Ca group ($P=0.07$). Also, the trials to criterion of the E2 and E2+P4 groups were lower than those of the OVX-normal-Ca and P4 groups, i.e. the OVX-normal-Ca and P4 groups satisfied the criterion in about 23 and 20 trials, respectively, while the E2 and E2+P4 groups satisfied it in about 14 and 13 trials, respectively. Furthermore, the number of trials to criterion was substantially higher in the OVX-low-Ca group than in the 17 β -estradiol-treated group, i.e. the LE2 group satisfied the criterion in about 16 trials, while the OVX-low-Ca group did not satisfy the criterion until about 28 trials. Additionally, the LP4 and LE2+LP4 groups did not satisfy the criterion until 25 trials. However, there were no significant differences between the OVX-low-Ca and LP4, and LP4 and LE2+LP4 groups.

As for the number of correct choices Fig. 3B, two-factorial ANOVA showed significant differences between normal- and low-Ca groups ($F(1,48)=6.99, P<0.05$), though the effect of hormonal treatment and the calcium diet \times hormonal treatment interaction were not significant. However, post hoc tests showed that there were tendencies

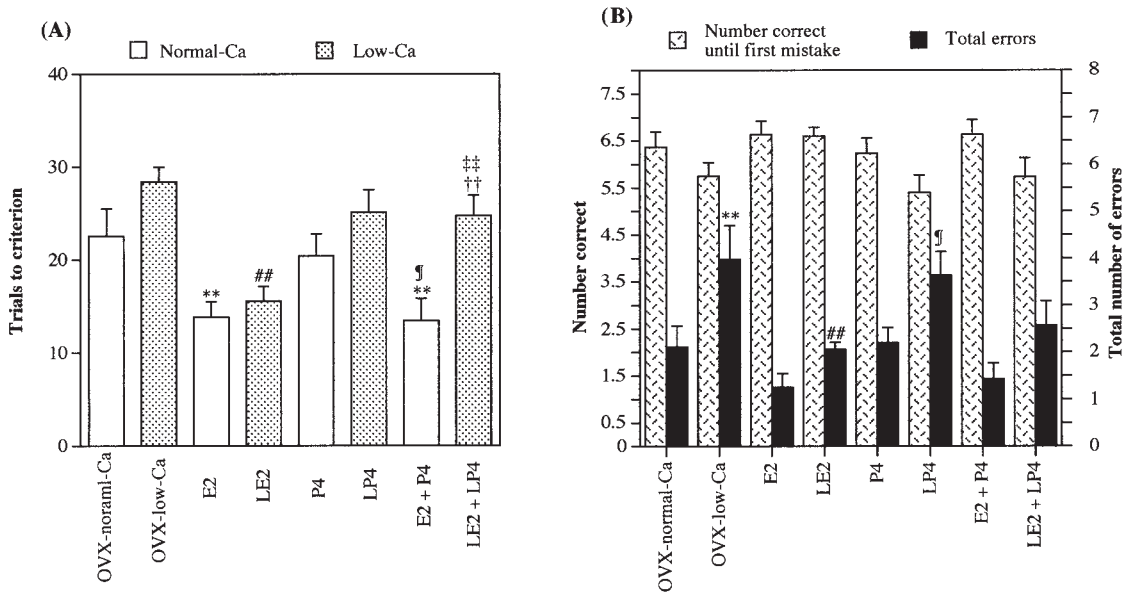


Fig. 3. The number of trials until the criterion was reached (A) and choice accuracy (B) in the regular trial in rats with normal- and low-calcium diets. The choice accuracy was evaluated by “number correct until first mistake” and “total number of errors.” The number correct until first mistake and the total number of errors were calculated as averages for the five consecutive trials until the performance criterion was reached. Results are expressed as the mean \pm S.E. M. ($n=7$ per group). Comparisons of changes among different treatment groups were made using two-factorial ANOVA with post hoc tests. ** $P<0.05$ vs. OVX-normal-Ca group; ## $P<0.01$ vs. OVX-low-Ca group; † $P<0.05$ vs. P4 group; †† $P<0.01$ vs. E2+P4 group; ††† $P<0.05$ vs. LE2 group.

toward to higher scores in the E2+P4 group versus the LE2+LP4 group ($P=0.052$) and in the LE2 group versus the LE2+LP4 group ($P=0.06$) in the number of correct choices. Similarly, as for the total errors, two-factorial ANOVA revealed significant differences for the calcium diets ($F(1,48)=17.33$, $P<0.01$) and the hormonal treatments ($F(3,48)=4.67$, $P<0.01$), while the calcium diet \times hormonal treatment interaction was not significant. Post hoc tests showed significant differences between the OVX-normal-Ca and OVX-low-Ca ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and P4 and LP4 ($P<0.05$) groups. The analysis also showed a tendency towards higher scores in the LE2+LP4 than in the E2+P4 group ($P=0.07$) in the total errors, although there were no significant differences between the OVX-low-Ca and LE2+LP4, and LP4 and LE2+LP4 groups. In short, treatment with 17 β -estradiol promoted task acquisition, while treatment with progesterone did not alter the facilitative effect of estradiol on task acquisition in rats fed a normal-calcium diet. Although the treatment with

progesterone alone did not affect the number of trials to criterion, the number correct until first mistake, or the total errors of the OVX-low-Ca group, progesterone treatment abolished the effect of estradiol on the performance in animals maintained on the low-calcium diet.

Fig. 4 shows the number of correct choices until the first mistake (Fig. 4A) and the total number of errors in a block (Fig. 4B) for rats consuming normal- and low-calcium diets. Each trial block consisted of six consecutive trials. As for the number of correct choices until the first mistake, two-factorial ANOVA revealed significant differences between normal- and low-calcium diets at blocks 3 ($F(1,48)=25.25$, $P<0.01$), 4 ($F(1,48)=42.3$, $P<0.01$), and 5 ($F(1,48)=40.51$, $P<0.01$), and among the hormonal treatments groups at blocks 2 ($F(3,48)=12.43$), 3 ($F(3,48)=9.60$, $P<0.01$), 4 ($F(3,48)=2.53$, $P<0.01$), and 5 ($F(3,48)=12.8$, $P<0.01$). There was a significant interaction of calcium diet \times treatment in block 5 only ($F(3,48)=3.47$, $P<0.05$). Although not statistically significant, a calcium diet \times hor-

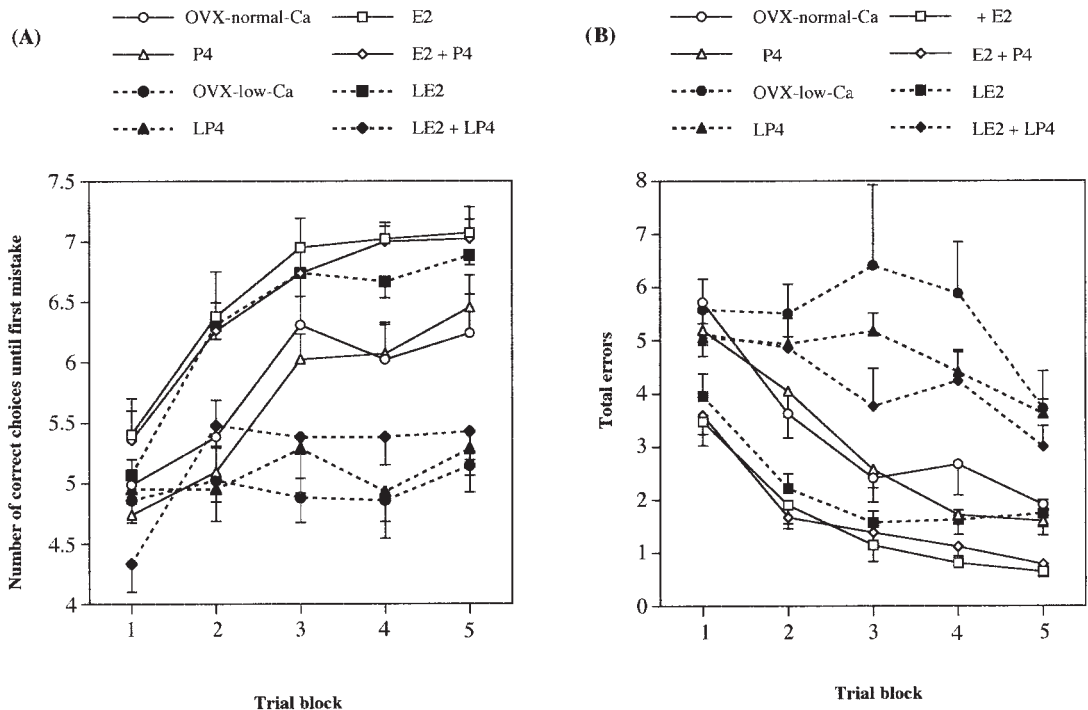


Fig. 4. Changes in the number of correct choices until the first mistake (A) and total number of incorrect choices (B) for maze performance of each hormonal treatment and diet group in rats. The values are the average number of correct choices until the first mistake \pm S.E.M. (A) and the average total number of error choices \pm S.E.M. (B) for seven rats per group. A block is the mean of six consecutive trials.

monal treatment interaction trend was observed for blocks 3 ($F(3,48)=2.37$, $P=0.08$) and 4 ($F(3,48)=2.53$, $P=0.07$). The results of statistical analysis indicated that the change in the number of correct choices until the first mistake differed for each treatment group during the regular trials (i.e. each treatment group differed in its ability to resolve the radial maze task). Furthermore, we compared each treatment group by post hoc tests on each trial block, and there were significant differences between the following groups: OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.05$), P4 and E2+P4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.05$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.05$) in block 2; OVX-normal-Ca and OVX-low-Ca ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 3; OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.01$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and E2+P4 ($P<0.01$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 4; and OVX-normal-Ca and E2 ($P<0.05$), OVX-normal-Ca and E2+P4 ($P<0.05$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 5. There were no significant differences between the OVX-normal-Ca and P4, E2 and E2+P4, OVX-low-Ca and LP4, OVX-low-Ca and LE2+LP4, and LP4 and LE2+LP4 groups in any trial block.

As for the data shown in Fig. 4B, two-factorial ANOVA revealed significantly different effects of the calcium diets in blocks 2 ($F(1,48)=26.7$, $P<0.01$), 3 ($F(1,48)=18.42$, $P<0.01$), 4 ($F(1,48)=41.16$, $P<0.01$), and 5 ($F(1,48)=47.71$, $P<0.01$) and of the hormonal treatments in blocks 1 ($F(3,48)=4.20$, $P<0.05$), 2 ($F(3,48)=15.26$, $P<0.01$), 3 ($F(3,48)=26.23$, $P<0.01$), 4 ($F(3,48)=10.82$, $P<0.01$), and 5 ($F(3,48)=8.13$, $P<0.01$). There was also a significant interaction of calcium diet \times treatment at block 2 ($F(3,48)=4.34$, $P<0.01$). Although not statistically significant, a calcium diet \times hormonal treatment trend was apparent for block 3 ($F(3,48)=1.80$, $P=0.10$) and 4 ($F(3,48)=2.14$, $P=0.11$). Furthermore, we also compared each treatment group by post hoc tests in each trial group, and found significant differences between the following groups: OVX-normal-Ca and E2 ($P<0.01$) and OVX-normal-Ca

and E2+P4 ($P<0.05$) in block 1; OVX-normal-Ca and E2 ($P<0.01$), OVX-normal-Ca and E2+P4 ($P<0.01$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and E2+P4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), and LE2 and LE2+LP4 ($P<0.01$) in block 2; OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and LP4 ($P<0.05$), E2+P4 and LE2+LP4 ($P<0.05$), OVX-low-Ca and LE2 ($P<0.01$), OVX-low-Ca and LE2+LP4 ($P<0.05$), and LE2 and LE2+LP4 ($P=0.05$) in block 3; OVX-normal-Ca and E2 ($P<0.05$), OVX-normal-Ca and E2+P4 ($P<0.05$), OVX-normal-Ca and OVX-low-Ca ($P<0.01$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$), OVX-low-Ca and LE2+LP4 ($P<0.05$), and LE2 and LE2+LP4 ($P<0.01$) in block 4; and OVX-normal-Ca and E2 ($P<0.05$), OVX-normal-Ca and E2+P4 ($P<0.05$), OVX-normal-Ca and OVX-low-Ca ($P<0.05$), E2 and LE2 ($P<0.05$), P4 and LP4 ($P<0.01$), E2+P4 and LE2+LP4 ($P<0.01$), OVX-low-Ca and LE2 ($P<0.01$, and LE2 and LE2+LP4 ($P<0.05$) in block 5.

3) Effect of steroid hormones on (Na⁺+K⁺)-ATPase activity inhibition-induced amnesia on the step-through passive avoidance task in gonadectomized mice³⁾.

To examine the possible roles and mechanism of action of steroid hormones against amnesia induced by ouabain, an inhibitor of (Na⁺, K⁺)-ATPase, gonadectomized male mice were administrated ouabain (0.1 μg per mouse) intracisternally (i.cist.), and the learning and memory abilities of the mice were assessed by a step-through passive avoidance task. Subcutaneous (s.c.) administration of 17 β -estradiol (E2; 10 μg kg^{-1}) or testosterone (TES; 1 mg kg^{-1}) improved the memory impairment induced by ouabain, while administration of dihydrotestosterone (1 mg kg^{-1}) or corticosterone (COR) (1 mg kg^{-1}) did not. Treatment with the estradiol receptor antagonists, tamoxifen (TAM) (10 mg kg^{-1} ; s.c. or 0.1 μg ; i.cist.) and 4-hydroxytamoxifen (10 mg kg^{-1} ; s.c.), or the androgen receptor antagonist, cyproterone (10 mg kg^{-1} ; s.c. or 1 μg ; i.cist.), did not influence the protective effect of E2 or TES on ouabain-induced amnesia. Moreover, we studied the effects of several free radical scavengers-17 β -estradiol (10 μg kg^{-1} ; s.c.), α -tocopherol (VE: 200 mg kg^{-1} ; per os (p.o.)), ascorbic acid (VC: 200 mg kg^{-1} ; p.o.), or VE+VC (200 mg kg^{-1} each; p.o.) on ouabain-induced amnesia, and compared those effects with that of E2. The

administration of free radical scavengers had no significant effect on memory impairment. These results indicate that

E2 and TES ameliorate the amnesia induced by inhibition of (Na⁺, K⁺)-ATPase activity, and that the protective effect of E2 is caused by a non-genomic, rather than a genomic effect or a radical scavenging action. Additionally, the ameliorative effect of TES does not appear to involve free radical scavenging, but its aromatization to estrogen could contribute to the non-genomic action of E2.

Above abstract was derived mainly from following experimental results²⁾.

Effects of steroid hormones on ouabain-induced amnesic and ouabain-untreated mice in the step-through passive avoidance test was determined. The effects of steroid hormones on ouabain-induced amnesia (Fig. 5A) differed significantly among the treated groups ($F(6,59) = 2.67$; $P < 0.05$). Post hoc test showed that pretreatment with E2 ($10 \mu\text{g kg}^{-1}$) significantly inhibited ($P < 0.01$) the ouabain-induced amnesic effect on latency time for the step through latency trial (STL). TES, a testicular steroid hormone, did not have a significant effect on ouabain-induced

amnesia at a dose of 0.1 mg kg^{-1} , but showed a significant protective effect at 1 mg kg^{-1} ($P < 0.05$). DHT, a non-aromatizable androgen, at doses of 0.1 and 1 mg kg^{-1} did not significantly inhibit ouabain-induced impairment of learning and memory. The administration of COR (0.1 or 1 mg kg^{-1}), an endogenous adrenocortical steroid hormone in rats and mice, did not have a significant effect on ouabain-induced amnesia. Additionally, the administration of E2 ($10 \mu\text{g kg}^{-1}$) and TES (1 mg kg^{-1}) did not have a significant effect in sham-operated or gonadectomized (GOX) -mice that were not treated with ouabain (Fig. 5B).

As shown in Fig. 5, TES at a dose of 1 mg kg^{-1} protected against ouabain-induced amnesia in the step-through passive avoidance task. Since the hypothalamus is capable of aromatizing a small proportion of testosterone to estradiol⁴⁰⁾ or androstenedione to oestrone⁴¹⁾, the protective effect of TES could result from its aromatization to an estrogen. This possibility was further suggested by the observation that dihydrotestosterone (DHT), a non-aromatizable androgen, did not protect against ouabain-induced amnesia (Fig. 5). To further address this possibility, we studied whether the protective effect of TES against ouabain-

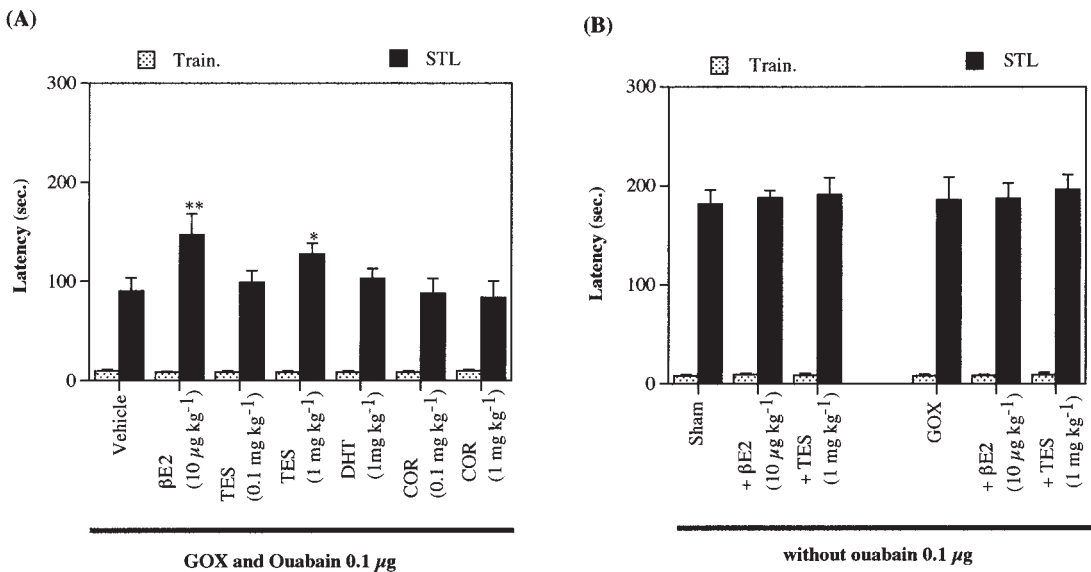


Fig. 5. Effects of steroid hormones on ouabain-induced amnesia (A) and on untreated Sham and GOX-mice in the step-through passive avoidance task. Steroid pretreatment was given 5 days before the Train. The mice were gonadectomized and injected intracisterally with $0.1 \mu\text{g}$ ouabain (A), or treated with same volume of vehicle without ouabain (B). Values are mean \pm S.E.M. for 5-10 animals per group. * $P < 0.05$; ** $P < 0.01$ vs. STL in the vehicle group (by post hoc test). E2=17 -estradiol, TES: testosterone, DHT: dihydrotestosterone, COR: corticosterone.

induced amnesia was mediated by androgen receptors.

We studied the influence of TAM (i.cist.) and 4-hydroxytamoxifen (HYT; s.c.) on the recovery from ouabain-induced memory impairment by E2 (Fig. 6). Although ANOVA revealed a significant difference between groups ($F(5,52)=2.44$; $P<0.05$), the following post hoc test showed that the treatment with TAM at 0.1 μg (i.cist.) did not affect the E2-induced improvement of latency time on the STL. In addition, HYT is a metabolite of tamoxifen, and is a more potent estrogen antagonist than TAM⁴². However, this more potent antagonist at a dose of 10 mg kg^{-1} (s.c.) did not inhibit the recovery from ouabain-induced amnesia caused by E2.

Fig.7 shows the influence of cyproterone, an

antiandrogen, on the protective effect of TES against ouabain-induced impairment of learning and memory in the step-through passive avoidance task. Cyproterone was given s.c. at a dose of 10 mg kg^{-1} , which was reported to antagonize the action of 1 mg kg^{-1} TES⁴³, and the i.cist. doses used were 0.1 and 1 μg per mouse. The s.c. or i.cist. administration of cyproterone alone did not affect ouabain-induced amnesia in the step-through passive avoidance task, and cyproterone administered s.c. or i.cist. in combination with TES did not significantly modify the protective action of TES against ouabain-induced amnesia. Cyproterone at a dose of 0.1 μg (i.cist.) also had no effect on ouabain-induced amnesia or the protective effect of 1 mg kg^{-1} TES.

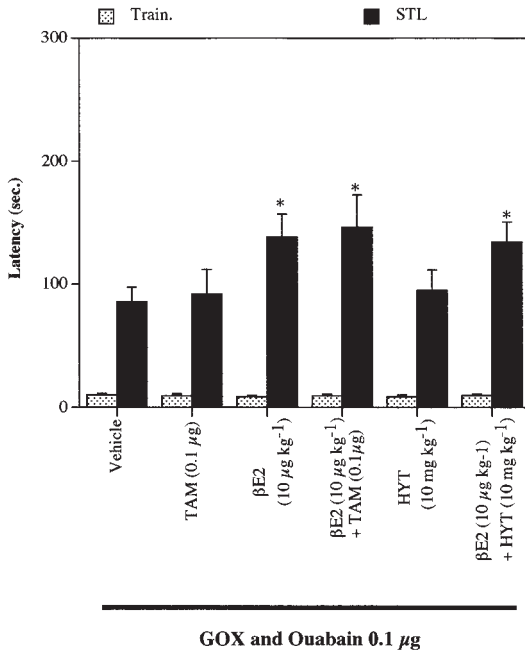


Fig. 6. Influence of TAM and HYT on the protective effect of E2 against ouabain-induced amnesia in the step-through passive avoidance task. All mice were gonadectomized, injected with 0.1 μg ouabain i.cist. and given either vehicle, E2 (10 $\mu\text{g kg}^{-1}$; s.c.), TAM (0.1 μg ; i.cist.), HYT (10 mg kg^{-1} ; s.c.), E2 +TAM, or E2+HYT. Neither TAM nor HYT influenced the latency time on the retention trial of E2-treated mice. Values are mean \pm S.E.M. for 8-12 animals per group. * $P<0.05$ vs. STL in the vehicle group (by post hoc test). E2=17 β -estradiol, TAM: tamoxifen, HYT: 4-hydroxytamoxifen.

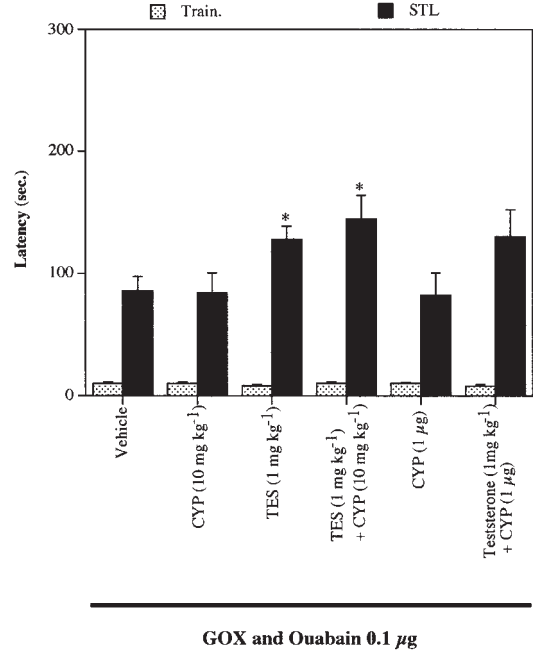


Fig. 7. Influence of cyproterone treatment on the protective effect of TES against ouabain-induced impairment of learning and memory in the step-through passive avoidance task. All mice were gonadectomized and injected with 0.1 μg ouabain i.cist. and then given TES (1 mg kg^{-1} ; s.c.), CYP (10 mg kg^{-1} ; s.c. or 1 μg ; i.cist.) or TES+CYP (either s.c. or i.cist.). Both doses of CYP failed to affect the recovery from ouabain-induced amnesia by TES. Values are mean \pm S.E.M. for 8-10 animals per group. * $P<0.05$ vs. STL in the vehicle group (by post hoc test). TES: testosterone, CYP: cyproterone.

As mentioned above, the protective effect of TES suggests that estradiol, which can be generated by the aromatization of TES, could affect ouabain-induced amnesia via estradiol receptors. We, therefore, examined the effect of TAM on the protective action of TES against ouabain-induced amnesia in the step-through passive avoidance task. As shown in Fig. 8, 0.1 μg TAM (i.cist.) did not influence the protective action of TES (1 mg kg^{-1}).

Conclusive remarks

As mentioned above, it seems that estradiol alone has a beneficial effect on the learning and memory, or mood formation. However, when estradiol was used together with progesterone, negative effects appear. Thus, neuroactive steroids may play important distinct roles in the regulation of the learning and memory, or the mood formation probably through their non-genomic or indirect action rather than a genomic action on their receptors, but the mechanisms behind their effects are not clear and must be wait for the

future research.

References

- 1) Sato T., Teramoto T., Tanaka K-I., Ohnishi Y., Irifune M., Nishikawa T.: Effects of ovariectomy and calcium deficiency on learning and memory of eight-arm radial maze in middle-aged female rats. *Behav. Brain Res.*, 142, 207-216, 2003.
- 2) Sato T., Tanaka K-I., Ohnishi Y., Teramoto T., Irifune M., Nishikawa T.: Effects of estradiol and progesterone on radial maze performance in middle-aged female rats fed a low-calcium diet. *Behav. Brain Res.*, 150, 33-42, 2004.
- 3) Sato T., Tanaka K-I., Ohnishi Y., Teramoto T., Irifune M., Nishikawa T.: Effects of steroid hormones on (Na^+ , K^+)-ATPase activity inhibition-induced amnesia on the step-through passive avoidance task in gonadectomized mice. *Pharmacol. Res.*, 49, 151-159, 2004.
- 4) Baulieu E.E., Robel P.: Neurosteroids; a new brain function? *J. Steroid Biochem. Mol. Biol.*, 37, 395-403, 1999.
- 5) Compagnone N.A., Mellon S.H.: Neurosteroids; biosynthesis and function of these novel neuromodulators. *Front. Neuroendocrinol.*, 21, 1-56, 2000.
- 6) Hu Z.Y., Jung-Testas I., Robel P., Baulieu E.E.: Neurosteroids; steroidogenesis in primary cultures of rat glial cells after release of aminoglutethimide blockade. *Biochem. Biophys. Res. Commun.*, 161, 917-922, 1989.
- 7) Jung-Testas I., Renoir M., Bugnard H., Greene G.L., Baulieu E.E.: Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. *J. Steroid Biochem. Mol. Biol.*, 41, 621-631, 1992.
- 8) Jurevics H., Morell P.: Cholesterol for synthesis of myelin is made locally, not imported into brain. *J. Neurochem.*, 64, 895-901, 1995.
- 9) Goascogne Le, Robel P., Gouezou M., Sananes N., Baulieu E.E., Waterman M.: Neurosteroids; cytochrome P-450scc in rat brain. *Science*, 237, 1212-1215, 1987.
- 10) Hojo Y., Hattori T.A., Enami T., Furukawa A., Suzuki K., Ishii H.T., Mukai H., Morrison J.H., Janssen W.G., Kominami S., Harada N., Kimoto T., Kawato S.: Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450

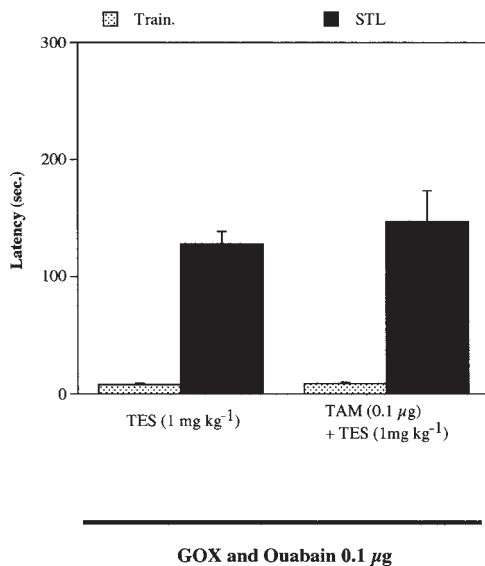


Fig. 8. The effect of TAM on TES-mediated protection against ouabain-induced amnesia in the step-through passive avoidance task. All mice were gonadectomized and injected with 0.1 μg ouabain i.cist., and given TES (1 mg kg^{-1} ; s.c.) or TES+TAM (0.1 μg ; i.cist.). TAM did not have a significant effect on the recovery from ouabain-induced amnesia by TES. Values are mean \pm S.E.M. for 8-10 animals per each group. TES: testosterone, TAM: tamoxifen.

- aromatase localized in neurons. *Proc. Natl. Acad. Sci. U. S. A.*, 101, 865-870, 2004.
- 11) Speroff L., Glass R.H., Kase N.G.: Clinical gynecologic endocrinology and infertility. In: Mitchell C., Editor (6th ed.), Lippincott Williams and Wilkins, 1999.
 - 12) Baker L., Meldrum K.K., Wang M., Sankula R., Vanam R., Raiesdana A., Tsai B., Hile K., Brown J. W., Meldrum D.R.: The role of estrogen in cardiovascular disease. *J. Surg. Res.*, 115, 325-344, 2003.
 - 13) Behl C.: Estrogen can protect neurons: modes of action. *J. Steroid Biochem. Mol. Biol.*, 83, 195-197, 2002.
 - 14) McEwen B.S.: Estrogens effects on the brain; multiple sites and molecular mechanisms. *J. Appl. Physiol.*, 91, 2785-2801, 2001.
 - 15) Walter P., Green S., Green G., Krust A., Bornert J.M., Jeltsch J.M., Staub A., Jensen E., Scraze G., Waterfield M.: Cloning of the human estrogen receptor cDNA. *Proc. Natl. Acad. Sci. U. S. A.*, 82, 7889-7893, 1985.
 - 16) Kuiper G.G., Gustafsson J.A.: The novel estrogen receptor-beta subtype; potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. *FEBS Lett.*, 410, 87-90, 1997.
 - 17) Ruff M., Gangloff M., Wurtz J.M., Moras D.: Estrogen receptor transcription and transactivation; Structure-function relationship in DNA- and ligand-binding domains of estrogen receptors. *Breast Cancer Res.*, 2, 353-359, 2000.
 - 18) Pettersson K., Grandien K., Kuiper G.G., Gustafsson J.A.: Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Mol. Endocrinol.*, 11, 1486-1496, 1997.
 - 19) Hart S.M.: Modulation of nuclear receptor dependent transcription. *Biol. Res.*, 35, 95-303, 2002.
 - 20) DeMarzo A.M., Beck C.A., Onate S.A., Edwards D.P.: Dimerization of mammalian progesterone receptors occurs in the absence of DNA and is related to the release of the 90-kDa heat shock protein. *Proc. Natl. Acad. Sci. U. S. A.*, 88, 72-76, 1991.
 - 21) Weiland N.G.: Estradiol selectively regulates agonist binding sites on the N-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology*, 131, 662-668, 1992.
 - 22) Paech K., Webb P., Kuiper G.G., Nilsson S., Gustafsson J., Kushner P.J., Scanlan T.S.: Differential ligand activation of estrogen receptors ER α and ER β at AP1 sites. *Science*, 277, 1508-1510, 1997.
 - 23) Kuiper G.G., Carlsson B., Grandien K., Enmark E., Haggblad J., Nilsson S., Gustafsson J.A.: Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*, 138, 863-870, 1997.
 - 24) Shughrue P.J., Lane M.V., Scrimo P.J., Merchenthaler, I.: Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. *Steroids*, 63, 498-504, 1998.
 - 25) Alves S.E., Weiland N.G., Hayashi B., McEwen B.S.: Immunocytochemical localization of nuclear estrogen receptors and progesterin receptors within the rat dorsal raphe nucleus. *J. Comp. Neurol.*, 391, 322-334, 1998.
 - 26) Osterlund M.K., Halldin C., Hurd Y.L.: Effects of chronic 17 β -estradiol treatment on the serotonin 5-HT (1A) receptor mRNA and binding levels in the rat brain. *Synapse*, 35, 39-44, 2000.
 - 27) Sherwin B.B., Tulandi T.: "Add-back" estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. *J. Clin. Endocrinol. Metab.*, 81, 2545-2549, 1996.
 - 28) Osterlund M.K., Gustafsson J.A., Keller E., Hurd Y.L.: Estrogen receptor beta (ER β) messenger ribonucleic acid (mRNA) expression within the human forebrain; distinct distribution pattern to ER α mRNA. *J. Clin. Endocrinol. Metab.*, 85, 3840-3846, 2000.
 - 29) Bethea C.L.: Colocalization of progesterin receptors with serotonin in raphe neurons of macaque. *Neuroendocrinology*, 57, 1-6, 1993.
 - 30) Bethea C.L., Widmann A.A.: Differential expression of progesterin receptor isoforms in the hypothalamus, pituitary, and endometrium of rhesus macaques. *Endocrinology*, 139, 677-687, 1998.
 - 31) Greco B., Allegretto E.A., Tetel M.J., Blaustein D.: Coexpression of ER beta with ER alpha and progesterin receptor proteins in the female rat forebrain; effects of estradiol treatment. *Endocrinology*, 142, 5172-5181, 2001.

- 32) Lee S.J., McEwen B.S.: Neurotrophic and neuroprotective actions of estrogens and their therapeutic implications. *Ann. Rev. Pharmacol. Toxicol.*, 41, 569-591, 2001.
- 33) Mattson M.P., Robinson N., Guo Q.: Estrogens stabilize mitochondrial function and protect neural cells against the pro-apoptotic action of mutant presenilin-1. *Neuro Report*, 8, 3817-3821, 1997.
- 34) Wang M., He Y., Eisenman L.N., Fields C., Zeng C. M., Mathews J., Ben A., Zorumski T., Fu E., Steinbach J.H., Covey D.F., Zorumski C.F., Mennerick S.: 3Beta-hydroxypregnane steroids are pregnenolone sulfate-like GABA (A) receptor antagonists. *J. Neurosci.*, 22, 3366-3375, 2002.
- 35) Wise P.M.: Estrogens and neuroprotection. *Trends Endocrinol. Metab.*, 13, 229-230, 2002.
- 36) Stromberg J., Haage D., Taube M., Bäckström T., Lundgren P.: Neurosteroid modulation of allopregnanolone and GABA effect on the GABA-A receptor. *Neuroscience*, 143, 73-81, 2006.
- 37) Adams M.M., Shah R.A., Janssen W.G., Morrison J.H.: Different modes of hippocampal plasticity in response to estrogen in young and aged female rats. *Proc. Natl. Acad. Sci. U. S. A.*, 98, 8071-8076, 2001.
- 38) Wang C., Christine E., Marx A., Morrow L., Wilkie A.: Neurosteroid modulation of GABAergic neurotransmission in the central amygdala: A role for NMDA receptors. *Neuroscience Letters*, 415, 118-123, 2007.
- 39) Woolley C.S., McEwen B.S.: Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J. Neurosci.*, 12, 2549-2554, 1992.
- 40) Weiz J., Gibbs C.: Conversion of testosterone and androstenedione to estrogens in vitro by the brain of female rats. *Endocrinology*, 94, 616-620, 1974.
- 41) Naftolin F., Ryan K.J., Petro Z.: Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. *Endocrinology*, 90, 295-298, 1972.
- 42) Kupfer D., Mani C., Lee C.A., Rifkind A.B.: Induction of tamoxifen-4-hydroxylation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), -naphthoflavone (-NF), and phenobarbital (PB) in avian liver; identification of P450 TCDDAA as catalyst of 4-hydroxylation induced by TCDD and -NF. *Cancer Res.*, 54, 3140-3144, 1994.
- 43) Roy E.J., Wade G.N.: Role of estrogens in androgen-induced spontaneous activity in male rats. *J. Comp. Physiol. Psychol.*, 89, 573-579, 1975.

麻酔薬による一酸化窒素 (NO) を介した 食道平滑筋弛緩反応の抑制機序

梶谷 淳

鹿児島大学大学院医歯学総合研究科 先進治療科学専攻
顎顔面機能再建学講座 歯科麻酔全身管理学分野

Mechanisms involving inhibition of NO-mediated lower esophageal sphincter relaxation by anesthetics

Atsushi Kohjitani

Department of Dental Anesthesiology, Field of Oral and Maxillofacial Rehabilitation,
Advanced Therapeutics Course,
Kagoshima University Graduate School of Medical and Dental Sciences
8-35-1 Sakuragaoka, Kagoshima 890-8544 Japan

Abstract

The enteric nervous system, along with extrinsic adrenergic and cholinergic innervations, play substantial roles in the regulation of gastrointestinal motility, secretion, blood flow, and the immune system by releasing various neurotransmitters, hormones and peptides. Nonadrenergic noncholinergic (NANC) nerves, one of the intrinsic innervations, are nitrenergic and mediate peristaltic waves, inhibitory responses, or relaxing mechanisms of the gastrointestinal tract, including the lower esophageal sphincter (LES). The LES has important physiological roles in allowing the passage of food and/or liquid in response to swallowing, while preventing gastroesophageal reflux by keeping the circular muscle contracted. LES contractility is one of the crucial factors in preventing regurgitation during general anesthesia. Isometric contraction model using rabbit LES showed that NANC relaxation was mediated by endogenously released nitric oxide (NO) from the myenteric plexus and that intravenous anesthetics ketamine and midazolam inhibit NANC relaxation via NO-3',5'-cyclic guanosine monophosphate (cGMP) pathway modulation. The inhibitory mechanisms for ketamine involved the generation of superoxide anions. Furthermore, the peripheral *N*-methyl-D-aspartate (NMDA) receptors in the myenteric plexus mediate NANC relaxation by modulating

extracellular production of superoxide anions, thus eliminating the relaxant effect of endogenous NO. Although the role of peripheral excitatory amino acids in the regulation of gastrointestinal motility under physiological and pathological conditions remains unclear, functional linkage of peripheral NMDA receptors in the myenteric plexus and the NO-cGMP pathway leading to smooth muscle relaxation could provide a key for elucidating the roles of endogenous NO in regulating gastrointestinal motility.

Key words: nitric oxide, nitric oxide synthase, NMDA receptor, gastrointestinal motility, lower esophageal sphincter, anesthetics

I. はじめに

高齢者では肺炎の罹患率が高く直接死因の第1位であり、その大部分は嚥下性肺炎であることが報告されている。嚥下性肺炎の原因として、(1)逆流胃内容物の誤嚥、(2)食物、水分の誤嚥、(3)病原性微生物を含む口腔・咽頭分泌物の反復微量吸引の3つがあり、これらのうち(3)の頻度が最も高いといわれている。起炎菌として、口腔内に常在する嫌気性菌群や *S. milleri* group を含む viridance streptococci で複数菌感染の形で検出される頻度が高いことは、口腔・咽頭分泌物の吸引を裏付けるものと考えられる。一方胃内容物、とくに胃液の誤嚥は chemical pneumonitis を惹起する。細胞浸潤による肺上皮組織の障害・壊死、血管透過性の亢進によって生じる肺間質や肺胞の浮腫は、成人呼吸窮迫症候群 (adult respiratory distress syndrome; ARDS) と呼ばれる重篤な障害をもたらすことが知られている。高齢者における胃液あるいは逆流胃内容物の誤嚥の頻度は、日本人の逆流性食道炎の高い罹患率からすると少なくないものと考えられる。

近年歯科領域でも嚥下機能改善のためのリハビリテーションや口腔衛生状態の改善に多くの努力が払われてきておりこれらの効果を一層高めるため、また歯科・口腔外科領域でより安全・快適で新しい全身麻酔法・静脈内鎮静法を開発するため、胃内容物の逆流防止機構すなわち胃食道接合部に位置する下部食道括約筋 (lower esophageal sphincter; LES) の収縮・弛緩機構に及ぼす調節因子に関する研究は重要である。LES は肥厚した特殊な平滑筋であり、生理的には嚥下運動に協調して食塊や水分を通過させる一方、通常は収縮し胃食道逆流を防いでいる。一般的に全身麻酔後には嚥下機能や気道防御機能が障害されることが知られており、LES 機能も影響を受ける可能性がある。一酸化窒素 (nitric oxide; NO) は蠕動運動、特に抑制性の反射および弛緩機構に関与することが知られている。したがって歯科・口腔外科領域で用いられる吸入麻酔薬

および静脈麻酔薬が、LES の内因性 NO を介する弛緩反応に及ぼす作用は重要である。一方、麻酔薬の中枢神経系における作用点として GABA_A (type A -amino butyric acid) 受容体や興奮性アミノ酸受容体の NMDA (*N*-methyl-D-aspartate) 型サブタイプが知られているが、末梢組織の NMDA 受容体に対する麻酔薬の作用は知られていない。本稿では各種全身麻酔薬の LES に対する直接作用および内因性 NO を介する弛緩反応に対する間接作用、ならびに筋層間神経叢に分布する末梢性 NMDA 受容体の役割について概説する。

II. 消化管平滑筋の運動調節機構

A. 消化管壁内神経系と非アドレナリン非コリン作動性 (NANC) 神経

消化管壁内神経系 (enteric nervous system; ENS) は、さまざまな神経伝達物質、消化管ホルモンおよび神経ペプチドを放出することにより、外因性支配神経であるアドレナリン作動性、コリン作動性神経とともに消化管運動、分泌、血流および免疫系の調節など広範囲の消化管機能に関与している^{1,2)}。LES の収縮・弛緩機構は、筋層間神経叢 (アウエルバツハ神経叢) に分布するさまざまなニューロン群から放出される神経伝達物質により、複雑な末梢性神経性調節を受けている。ウサギ LES におけるペプチド作動性神経が収縮・弛緩に及ぼす役割については、すでに報告した³⁾。

非アドレナリン非コリン作動性 (nonadrenergic noncholinergic; NANC) 神経は ENS を構成する神経支配のひとつであるが、消化管においては NO 作動性であり、蠕動運動とりわけ抑制性の反射および弛緩機構に重要な役割を果たしていることが知られている^{4,5)}。ヒトに非特異的 NO 阻害薬を投与すると、嚥下に引き続いて発生する食道体部・LES の協調的な弛緩反応 (蠕動波) が阻害されたとの報告⁶⁾があり、NO は嚥下に関連した生理的な食道弛緩反応の調節に重要な役割を果たすと考えられている。

B. NO のシグナル伝達と運動調節

NO は、現在もっとも注目されている神経伝達物質のひとつである。NO は、cytochrome P-450 に類似した NO 合成酵素 (nitric oxide synthase; NOS) により、基質である L-arginine を L-citrulline に転換する過程で合成されるラジカル状のシグナル伝達分子である⁷⁾。神経型 NO 合成酵素 (neuronal NOS; nNOS) 由来の NO は消化管において NANC 平滑筋弛緩因子であることが知られており、可溶性グアニル酸シクラーゼ (soluble guanylate cyclase; sGC) を刺激してサイクリック GMP (3', 5'-cyclic guanosine monophosphate; cGMP) を産生する。cGMP はさらにプロテインキナーゼ G (protein kinase G; PKG) およびその下流に位置するリン酸化カスケードを活性化し、この活性化の過程は最も特徴的で広く知られてきた NO のシグナル伝達経路である⁸⁾。LES においても NANC 弛緩反応が L-arginine-NO のシグナル伝達経路を介することがすでに明らかとなっている^{9,10)}。

血管平滑筋において、NO はスーパーオキシドと直ちに反応して peroxynitrite (ONOO⁻) を産生することによりその血管弛緩作用を失う^{11,12)}。事実は、スーパーオキシドが血管内皮由来弛緩因子 (endothelium-derived relaxing factor; EDRF) の不安定化に貢献することを示している¹²⁾。消化管においては、NO 作動性神経から放出される神経伝達物質 (nitroergic transmitter) は、NO を放出する分子であることが知られている^{13,14)}。このことはさまざまなスーパーオキシド産生薬が外因性 NO による弛緩反応を抑制するものの、内因性 NO を介した弛緩反応にはほとんど影響しないことから明らかになった。この nitroergic transmitter の安定性を説明する仮説として、これらが高レベルの組織 SOD (Cu/Zn superoxide dismutase; Cu/Zn SOD) で保護されている¹⁵⁾という考え方が受け入れられている。その理由として、diethyldithiocarbamic acid (DETCA) を用いて Cu/Zn SOD を失活させると内因性 NO がスーパーオキシドによって失活されやすくなる¹⁵⁾ことがある。LES における Cu/Zn SOD の果たす役割については未だ明らかではないが、これまでの報告では Cu/Zn SOD は正常食道運動の調節に関与しているようである¹⁶⁾。

Ⅲ. 興奮性アミノ酸受容体を介した神経伝達と NO 産生機序

A. 中枢神経系における NO 合成とその役割

中枢神経系においては、興奮性アミノ酸受容体の中

でも NMDA 型サブタイプが、グルタミン酸を介した神経伝達ならびにアセチルコリンやノルアドレナリンを含む他の神経伝達物質の産生に重要な役割を果たしている。NMDA 受容体と NOS が機能的にリンクしていることは良く知られている。グルタミン酸が NMDA 受容体に結合して受容体が活性化すると、すでに NO と同一物質であることが証明されている EDRF が放出される¹⁷⁾。中枢神経系における NO の機能的特異性は、グルタミン酸が L-arginine から L-citrulline への転換および cGMP 産生を刺激し、また NOS 阻害薬が酵素活性および cGMP 産生をとともに抑制したという報告¹⁸⁻²¹⁾に由来する。したがって、ある一定の生理的条件下で中枢神経系において NMDA 受容体の神経伝達が NO 合成を活性化することは明らかである。また NO 合成に対する麻酔薬の効果に関しては、すでに Gonzales らが大脳皮質ニューロンにおいて、ケタミン、NMDA 受容体のアンタゴニスト MK801、非特異的 NOS 阻害薬 L-monomethyl-L-arginine (L-NMMA) が NMDA 受容体を介した cGMP 産生を抑制することを報告している²²⁾。一方で、脳卒中・外傷・クモ膜下出血や虚血性脳障害などの急性神経退行性障害 (acute neurodegeneration) または筋萎縮性側索硬化症 (amyotrophic lateral sclerosis; ALS) やアルツハイマー病などの慢性神経退行性障害 (chronic neurodegeneration) の病因としては、グルタミン酸受容体、とりわけ NMDA 型サブタイプが過度に活性化され、nNOS の刺激を介して NO が大量に産生されることが関与する²³⁻²⁵⁾。

B. 消化管に分布する興奮性アミノ酸受容体の役割

興奮性アミノ酸受容体の存在は末梢組織においても知られている。グルタミン酸またはグルタミン酸を介した神経伝達は ENS だけでなく²⁶⁾、末梢の侵害受容²⁷⁾、気道収縮性の調節²⁸⁾、静脈の自動運動²⁹⁾、骨生成および維持³⁰⁾、膵島におけるグルカゴンおよびインスリンの分泌³⁰⁾にも関連することが報告されている。消化管においては、NMDA 受容体の免疫組織活性が guinea pig の粘膜下・筋層間神経叢³⁰⁾およびラット食道神経叢³¹⁾で証明されている。また guinea pig 回腸の筋層間神経叢における興奮性アミノ酸受容体は、NMDA 型サブタイプと類似した薬理的性質を持つことが報告されている^{32,33)}。これらの末梢の受容体はグルタミン酸および NMDA で活性化されて平滑筋収縮を生じ、Mg²⁺ イオンおよびテロドトキシンの存在下で不活化され、競合的アンタゴニスト DL-2-amino-5-phosphonovaleric

acid (AP5)^{32,34} および非競合的アンタゴニスト 5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5, 10-imine (MK801)³⁵ の存在下で拮抗される。しかし、筋層間神経叢において興奮性アミノ酸およびその受容体が消化管運動に及ぼす役割についてはほとんど報告がない。guinea pig 回腸の筋層間神経叢では、高カリウムで誘発した脱分極、フィールド電気刺激および神経節刺激薬の投与によりグルタミン酸の放出が増加した³⁶との報告がある。また guinea pig 結腸においてグルタミン酸および NMDA で NMDA 受容体を刺激すると、蠕動運動が抑制され、この抑制は AP5 で拮抗

された³⁷との報告がある。

IV. 麻酔薬による NANC 弛緩反応の修飾と末梢性 NMDA 受容体の関与

A. 静脈麻酔薬 (ケタミン, ミダゾラム)

著者らはこれまでにウサギ LES の等尺性張力変化モデルを用いて、LES を直接弛緩させるには比較的高濃度 ($10^{-4} \sim 10^{-3}$ M) のケタミンを必要とし、この弛緩反応は細胞外 Ca^{2+} の流入阻害およびアデニル酸シクラーゼ活性の増強を介することを報告している³⁸。この麻酔薬濃度は臨床使用濃度とは乖離してお

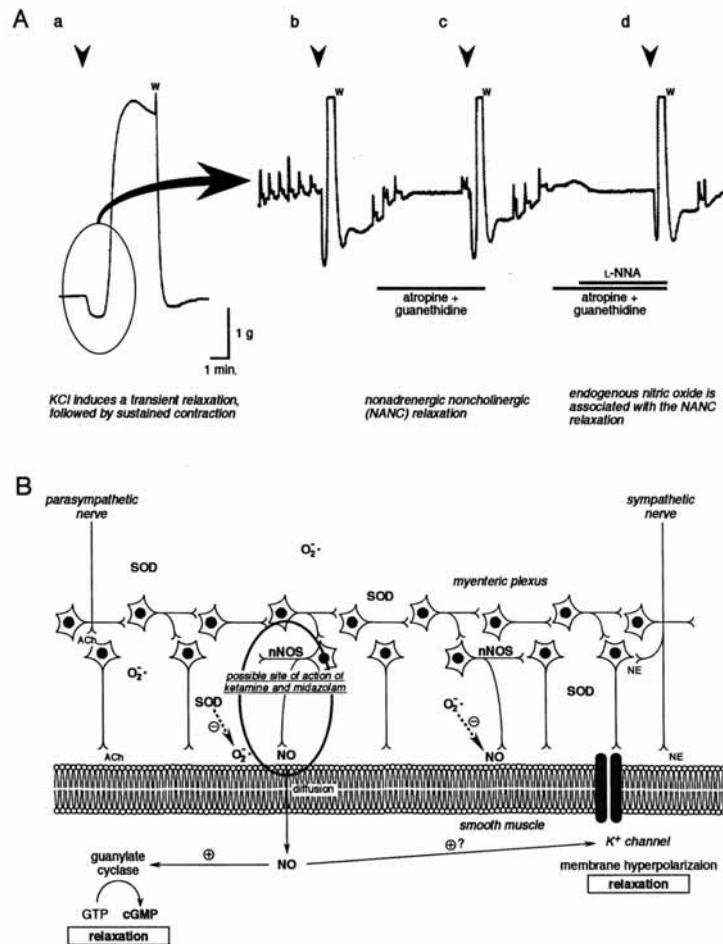


図 1 (A) ウサギ下部食道括約筋 (LES) においてアトロピン, グアネチジンの存在下にカリウム刺激で誘発した非アドレナリン非コリン作動性 (NANC) 弛緩反応。NANC 弛緩反応は非特異的 NOS 阻害薬 L-NNA で抑制された。(B) 消化管平滑筋とコリン作動性, アドレナリン作動性神経および筋層間神経叢の関係と NO の細胞内シグナル伝達。(Kohjitani A. et al. 2003⁴⁰から)

り、平滑筋弛緩はケタミンの非特異的作用と考えられる。静脈麻酔薬の間接的作用については、同様のモデルを用いて、まずカリウム刺激で誘発した早期の弛緩反応が筋層間神経叢の NOS の活性化を介した NO による NANC 弛緩反応であること (図 1 A) を明らかにし³⁹⁾、この弛緩反応に対する静脈麻酔薬の影響について検討した。ケタミン (図 2 A) およびミダゾラム (図 2 B) は NANC 弛緩反応を濃度依存的に抑制する (ケタミン: $EC_{50} = 8.8 \times 10^{-5} M$, ミダゾラム: $EC_{50} = 4.8 \times 10^{-6} M$) とともに筋肉中 cGMP 濃度を減少させること、ミダゾラムは NOS を介さない弛緩反応である diethylamine NONOate (DEA-NO) による弛緩反応に影響しない (図 2 H) が、ケタミンはこれを著明に抑制する (図 2 G) ことを見出した。この結果から、ケタミンおよびミダゾラムは NO - cGMP 系の調節を介して LES の NANC 弛緩反応を抑制することが明らか

かになった³⁹⁾。これら静脈麻酔薬の作用部位として、(1)筋層間神経叢において神経伝達を阻害し (テトロドトキシンのように) NO を含む神経伝達物質が減少・枯渇した、(2)筋層間神経叢において nNOS など NO 産生過程を抑制した、(3)スーパーオキシドの産生を介して NO を消去あるいは失活させた、あるいは(4) cGMP の分解を促進した可能性が示唆された (図 1 B)。これら静脈麻酔薬の作用部位についてさらに検討を行った結果、ケタミンとミダゾラムでは NANC 弛緩反応の抑制機序が全く異なることが明らかになった。ケタミンによる NANC 弛緩反応の抑制は、スーパーオキシドから過酸化水素と酸素への分解を触媒する酵素 SOD (superoxide dismutase; SOD) の前処置で部分的に回復した (図 2 D) が、過酸化水素から水と酸素への分解を触媒する酵素 catalase では回復しなかった。これに対して、ミダゾラムによる NANC 弛緩反

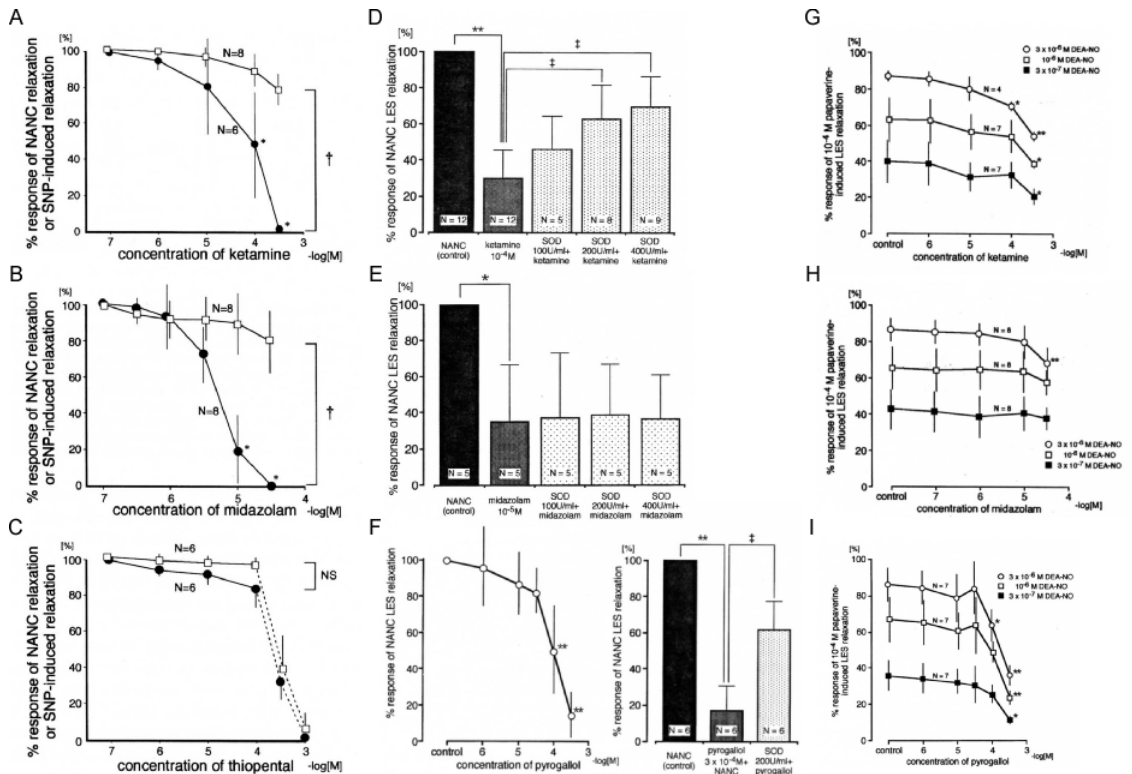


図 2 (A)ケタミン (B)ミダゾラム (C)チオペンタールによる NANC 弛緩反応抑制の用量反応曲線。(D)ケタミン (E)ミダゾラムによる NANC 弛緩反応抑制に対する SOD の効果。(F)スーパーオキシド産生薬 pyrogallol による NANC 弛緩反応抑制の用量反応曲線および SOD の効果。(G)ケタミン (H)ミダゾラム (I)pyrogallol が、外因性 NO ドナー diethylamine NONOate による弛緩反応におよぼす効果。(Kohjitani A. et al. 2001³⁹⁾, 2003⁴⁰⁾から)

応の抑制は SOD の前処置により回復しなかった (図 2 E)。ケタミンはさまざまな外因性 NO ドナー (DEA-NO, S-nitrosoglutathione, sodium nitroprusside) による弛緩反応も濃度依存的に抑制した。また興味あることに、スーパーオキシド産生薬 pyrogallol は NANC 弛緩反応および外因性 NO ドナーによる弛緩反応に対してケタミンと全く同様の挙動を示した。すなわち、NANC 弛緩反応を濃度依存的に抑制し (図 2 F 左)、pyrogallol による NANC 弛緩反応の抑制が SOD の前処置によって回復し (図 2 F 右)、DEA-NO による弛緩反応を濃度依存的に抑制した (図 2 I)。これらの所見から、ケタミンはスーパーオキシドの産生による内因性 NO の失活を介して LES の NANC 弛緩反応を抑制することが明らかとなった⁴⁰⁾。他方ミダゾラムは nNOS 活性を濃度依存的に抑制し、これにより NANC 弛緩反応を抑制することが明らかとなった⁴⁰⁾。

B. 吸入麻酔薬 (セボフルラン)

7%セボフルラン通気によるクレブス緩衝液中のセボフルラン濃度は、5分後0.911 minimum alveolar concentration (MAC), 10分後1.906 MAC, 15分後1.778 MAC, 20分後1.977 MAC であり、投与開始後10分経過すればセボフルランは十分飽和するものと考えられた。セボフルランは濃度依存的に筋肉片を弛緩させ、濃度依存的に NANC 弛緩反応を抑制した。臨床的に使用される濃度 (7%) で最大弛緩に近い弛緩を生じていることは注目される所見である。セボフルランによる内因性 NO を介した弛緩反応の抑制機序については明らかではないが、その作用点は今後さらに検討してゆく必要がある。

C. NANC 弛緩反応における末梢性 NMDA 受容体の役割

NMDA 受容体は受容体 - イオンチャネル複合体である。グルタミン酸または NMDA のようなアゴニストが受容体コンポーネントの認識部位に結合すると、イオンチャネルが開口し細胞外からの Na^+ および Ca^{2+} のコンダクタンスを上昇させる⁴¹⁾。MK801 は phencyclidine や他の phencyclidine 関連薬と同様に NMDA 受容体のイオンチャネルコンポーネントに結合し非競合的拮抗作用を示すアンタゴニストである⁴¹⁾。著者らの研究⁴²⁾では、MK801は通常のクレブス緩衝液中では NANC 弛緩反応を濃度依存的に抑制する (図 3 A, open circles) とともに、筋肉中 cGMP 濃度を減少させた (図 3 B, open columns)。NMDA 受容体のア

ゴニストである NMDA を投与すると、cGMP 濃度の上昇をともなって (図 3 D, open columns) 筋肉片を弛緩させた (図 3 C, open circles)。さらに、NMDA による cGMP 濃度の上昇は、非特異的 NO 阻害薬である N^G -nitro-L-arginine (L-NNA) によって抑制された (図 3 D, hatched columns)。これらの所見は末梢に存在する NMDA 受容体を刺激することにより、NOS が活性化し基質である L-arginine を L-citrulline に転換する過程で NO が産生され、cGMP が蓄積して平滑筋を弛緩させたことを示している。著者らはすでに NMDA 拮抗薬でもあるケタミンが NANC 弛緩反応を抑制し、同時に cGMP の産生を抑制することを観察しているが³⁹⁾、これは今回の結果と合致する所見である。また 1b 群抗不整脈薬であるメキシレチンが同様の反応を示すことを報告している⁴³⁾が、これは筋層間神経叢における神経伝達の阻害が関連しているものと考えられる。

Mg^{2+} イオンはイオンチャネルコンポーネントに非競合的に拮抗するため、 Mg^{2+} イオンを枯渇させると Ca^{2+} イオンの透過性が亢進し受容体機能に影響する可能性がある。著者らの研究⁴²⁾では、 Mg^{2+} を枯渇したクレブス緩衝液中において NANC 弛緩反応は有意に増強し、しかも筋肉中 cGMP 濃度の増加をともなっていた (図 3 B, closed column at control)。 Mg^{2+} を枯渇したクレブス緩衝液中において、MK801 による NANC 弛緩反応抑制の用量反応曲線は、通常のクレブス緩衝液中の用量反応曲線 ($\text{EC}_{50} = 1.5 \times 10^{-5} \text{ M}$) と比較して右方移動した (NANC 弛緩反応は抑制されにくくなった) ($\text{EC}_{50} = 7.4 \times 10^{-5} \text{ M}$) (図 3 A, closed circles)。この所見は、イオンチャネルコンポーネントを阻害する Mg^{2+} イオンがほとんど消失したため、 Ca^{2+} コンダクタンスを抑制するためにはより高濃度の NMDA アンタゴニストが必要になったものと考えられる。 Mg^{2+} イオンが他の生理的機能に影響している可能性は除外できないが、これらの所見は Mg^{2+} イオンが NMDA 受容体を介した NOS の活性化に何らかの役割を果たしていることを示している。

D. NANC 弛緩反応の抑制におけるスーパーオキシドの役割

著者らは NANC 弛緩反応の抑制過程におけるスーパーオキシドの役割を検討した⁴²⁾。その結果、SOD (100 U/ml) を前処置すると MK801 による NANC 弛緩反応抑制の用量反応曲線は右方移動した (MK801 による NANC 弛緩反応の抑制が回復した) ($\text{EC}_{50} = 3.4 \times$

10^{-5} M) が, catalase (100 U/ml) の前処置では変化しなかった。この所見は, スーパーオキシドが MK801 による NANC 弛緩反応の抑制に関与することを示唆している。次に DETCA 処理によって Cu/Zn SOD 活性を不活化すると, MK801 (図 3 G) による NANC 弛緩反応抑制の用量反応曲線は左方移動した (NANC 弛緩反応は抑制されやすくなった)。この所見は, Cu/Zn SOD の不活化によって内因性 NO のスーパーオキシドに対する感受性が増加して消去されやすく

なったため, 内因性 NO による NANC 弛緩反応が減弱したものと考えられる。

以上のことから, MK801 の存在下でカリウム刺激によって発生したスーパーオキシドが NANC 弛緩反応の抑制に関与したものと考えられた。Cu/Zn SOD の不活化が, スーパーオキシド産生薬である pyrogallol による NANC 弛緩反応抑制の用量反応曲線を左方移動させた (図 3 E) ことは, この結論を支持するものである。同様に, 著者らは Cu/Zn SOD を不

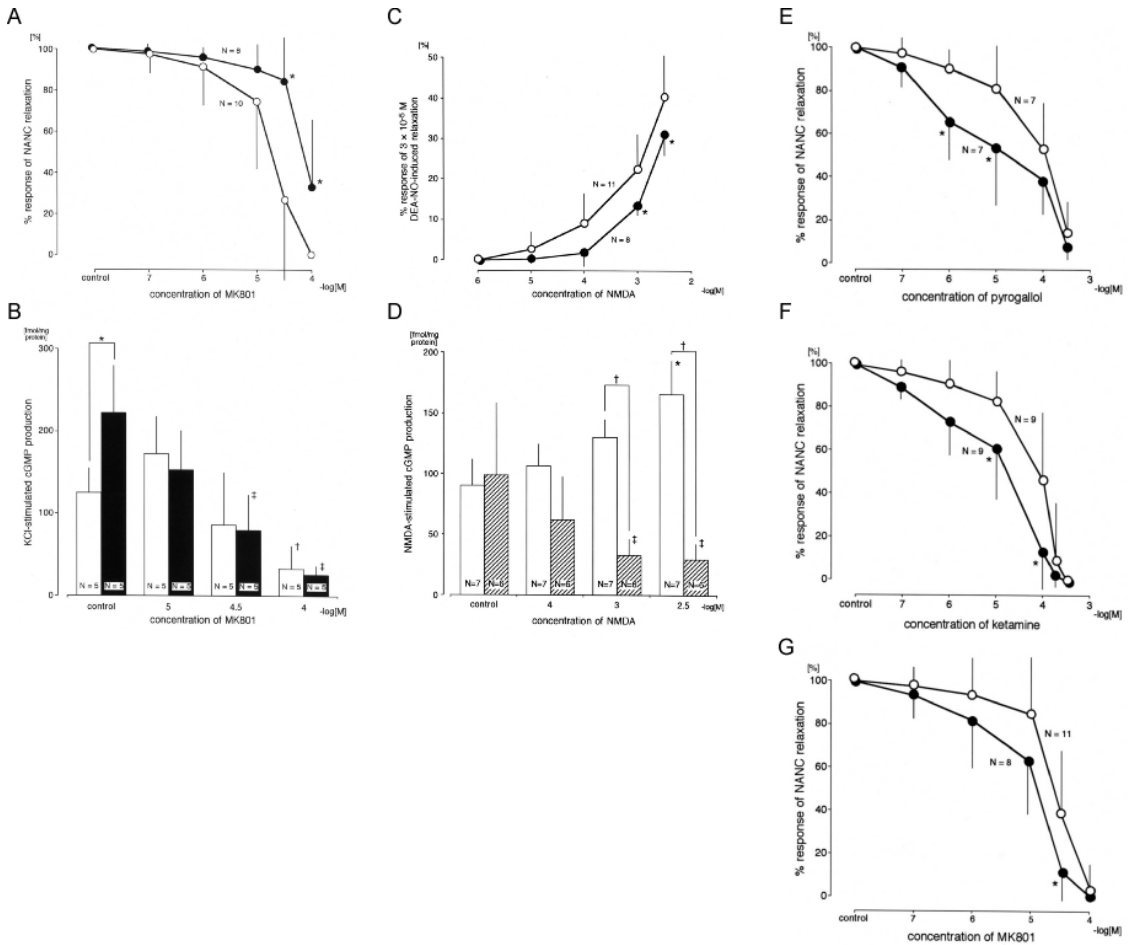


図 3 (A) NMDA 受容体アンタゴニスト MK801が, クレブス緩衝液中 (open circles) および Mg²⁺ 枯渴クレブス緩衝液中 (closed circles) において NANC 弛緩反応におよぼす効果。(B) MK801存在下で, クレブス緩衝液中 (open bars) および Mg²⁺ 枯渴クレブス緩衝液中 (closed bars) でカリウム刺激したときの筋肉中 cGMP 濃度の変化。(C) NMDA 刺激による LES 弛緩反応の用量反応曲線 (open circles) および MK801の効果 (closed circles)。(D) NMDA 刺激時の筋肉中 cGMP 濃度の変化 (open bars) および NOS 阻害薬 L-NNA の効果 (hatched bars)。(E) pyrogallol (F) ケタミン (G) MK801による NANC 弛緩反応抑制の用量反応曲線 (open circles) および Cu/Zn SOD 不活化 (closed circles) の効果。(Kohjitani A. et al. 2005⁴²⁾ から)

活化することによって、NMDA 拮抗薬であるケタミンによる NANC 弛緩反応抑制の用量反応曲線も左方移動した(図 3F)ことを報告している⁴⁰⁾。この所見は、ケタミンがスーパーオキシドの産生を介して NANC 弛緩反応を抑制したことを示唆するものであるが、これも今回の実験結果と合致した所見である。

消化管平滑筋における NO 合成とその役割

以上の研究結果から、ウサギ LES においては、末梢の NMDA 受容体にアゴニストが結合すると、中枢神経系ですでに報告されているのと同様に NOS が活性化される^{18, 21)}。筋層間神経叢の NMDA 受容体は NO-cGMP 経路を活性化することにより、平滑筋の NANC 弛緩反応の調節に重要な役割を果たしていると考えられる。これまでのグルタミン酸、NMDA またはそれらの受容体と消化管平滑筋の NANC 弛緩反応の関連では、意識下のイヌ⁴¹⁾およびボランティア⁴⁵⁾における報告がある。これらの報告は *in vivo* で NMDA 受容体アンタゴニストを投与しており、その作用部位は明らかではなかった。著者らの *in vitro* の研究⁴²⁾は、末梢の NMDA 受容体が迷走神経の求心性線維とは独立して nitrenergic な弛緩反応を調節することを初めて示唆したものである。

末梢組織における興奮性アミノ酸が生理的・病的状態において消化管運動の調節にどのように関与するかについては未だ明らかではないが、筋層間神経叢の末梢性 NMDA 受容体と NO-cGMP 経路を介したシグナル伝達が機能的に連携して消化管平滑筋の弛緩をもたらしていることは、近い将来消化管運動の調節における内因性 NO の役割を明らかにする上で重要な鍵になることは間違いない。興味あることに、局所的に産生された NO は消化管の麻痺をもたらす重要なメディエータであることが示唆されている⁴⁶⁾。Kalf らはラットを用いた研究で、消化管の操作により誘導された誘導型 NOS (iNOS) 由来の NO が、消化管操作後の消化管運動を著明に抑制することを報告している⁴⁷⁾。彼らの研究において、iNOS の発現における末梢性 NMDA 受容体の関与については明らかではないが、グルタミン酸が脳虚血後の iNOS 発現に貢献していることが報告されており⁴⁸⁾、消化管の末梢性 NMDA 受容体が術後イレウスの病因に関わっている可能性がある。

VI. 全身麻酔下での消化管機能への影響

A. ケタミン

ケタミンは LES 圧を上昇させる麻酔薬にも下降させる麻酔薬にも分類されていない⁴⁹⁾。著者らの知見は、LES において麻酔薬が末梢性 NMDA 受容体を介して NO 作動性の弛緩反応を変調させることにより、消化管運動に影響を与える可能性を示唆している。これまでにケタミン単独あるいはケタミンと他の麻酔薬との併用が消化管機能に与える影響について、全静脈麻酔下あるいは鎮静下においていくつかの研究がなされてきた。それらによると、鎮静量 (0.5 mg/kg 筋肉内投与) のケタミンは健常ボランティアにおいて胃排出に影響を与えず⁵⁰⁾、また小児に対しては、1 mg/kg 静脈内投与または 7-10 mg/kg 筋肉内投与の投与量で内肛門括約筋の収縮頻度、振幅、リズムカルな発射活動に影響しない⁵¹⁾ことが報告されている。これらはケタミンの消化管運動における望ましい作用であるが、一方プロポフォルとケタミンを併用した全身麻酔下 (1.4 mg/kg, 持続静脈内投与) では、プロポフォル単独あるいはイソフルラン単独の全身麻酔と比較すると、胃・盲腸における通過時間を延長させるようである⁵²⁾。またケタミンとプロポフォルを組み合わせた鎮静において、ケタミンの投与量を増加させていくと (0.5, 0.8 and 1.2 mg/kg, 持続静脈内投与)、術後の嘔気・嘔吐 (postoperative nausea and vomiting; PONV) の頻度が増加することが報告されている⁵³⁾。これらの報告された臨床所見が末梢性 NMDA 受容体の拮抗を介したものと判断することは困難であるが、ケタミンは総じて上部消化管運動を減弱させ、高用量では PONV の頻度を上昇させるようである。消化管運動の調節における末梢性 NMDA 受容体の役割を明らかにするためには、さらに臨床研究が必要になると思われる。

B. 吸入麻酔薬

吸入麻酔薬が消化管内圧におよぼす影響を検討した報告はほとんどない。著者らは全身麻酔中の小児において、吸入麻酔薬セボフルランとエンフルランの上部消化管内圧におよぼす影響 (直接作用) を検討した⁵⁴⁾。麻酔導入後上部消化管内圧センサーを挿入し、下部食道・LES・胃の内圧を 0~2.5 MAC における吸入麻酔薬の濃度下で連続的に測定した。両吸入麻酔薬は濃度依存的に LES 圧を軽度低下させた (図 4B) が、食道・胃の内圧は変化しなかった (図 4A, C)。胃・食道内圧較差 (barrier pressure) はエンフルラン群で有意な低下をみとめた (図 4D)。

Ⅶ. おわりに

ウサギ LES において内因性 NO を介する NANC 弛緩反応は、筋層間神経叢に分布する興奮性アミノ酸受容体の NMDA 型サブタイプを介した反応であることが明らかになった。NANC 弛緩反応は、部分的に細胞外におけるスーパーオキシド産生によって調節を受けており、これにより内因性 NO による弛緩作用が減弱する。ケタミンは末梢性 NMDA 受容体を拮抗することにより、NANC 弛緩反応を抑制する可能性が示唆された。吸入麻酔薬セボフルランは NANC 弛緩反応を著明に抑制した。

今回の著者らの知見は、吸入麻酔薬を含む各種麻酔薬が末梢性 NMDA 受容体を介して内因性 NO 作動性の弛緩反応を変調させることにより、消化管運動に影響を与える可能性を示唆している。消化管運動の調節における興奮性アミノ酸の役割については未だ明らかではないが、筋層間神経叢の NMDA 受容体と NO - cGMP 経路を介したシグナル伝達が機能的に連携して LES の弛緩をもたらす事実は、近い将来消化管運動

の調節における内因性 NO の役割を明らかにする上で重要な鍵になると期待される。

文 献

- 1) Guiton, A., Hall, J.: General principles of gastrointestinal function - motility, nervous control, and blood circulation., In: Textbook of Medical Physiology, 10th Edition. Guiton, A., Hall, J., eds. 718-727, W. B. Saunders Company, Philadelphia, 2000
- 2) Hansen, M. B.: The enteric nervous system II: gastrointestinal functions. Pharmacol Toxicol 92, 249-257, 2003
- 3) Kohjitani, A., Shirakawa, J., Okada, S., Obara, H.: Effects of various peptides on isolated rabbit lower esophageal sphincter. Peptides 17, 927-931, 1996
- 4) Sanders, K. M., Ward, S. M.: Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. Am J Physiol 262, G379-392, 1992
- 5) Rand, M. J.: Nitrgergic transmission: nitric oxide as a

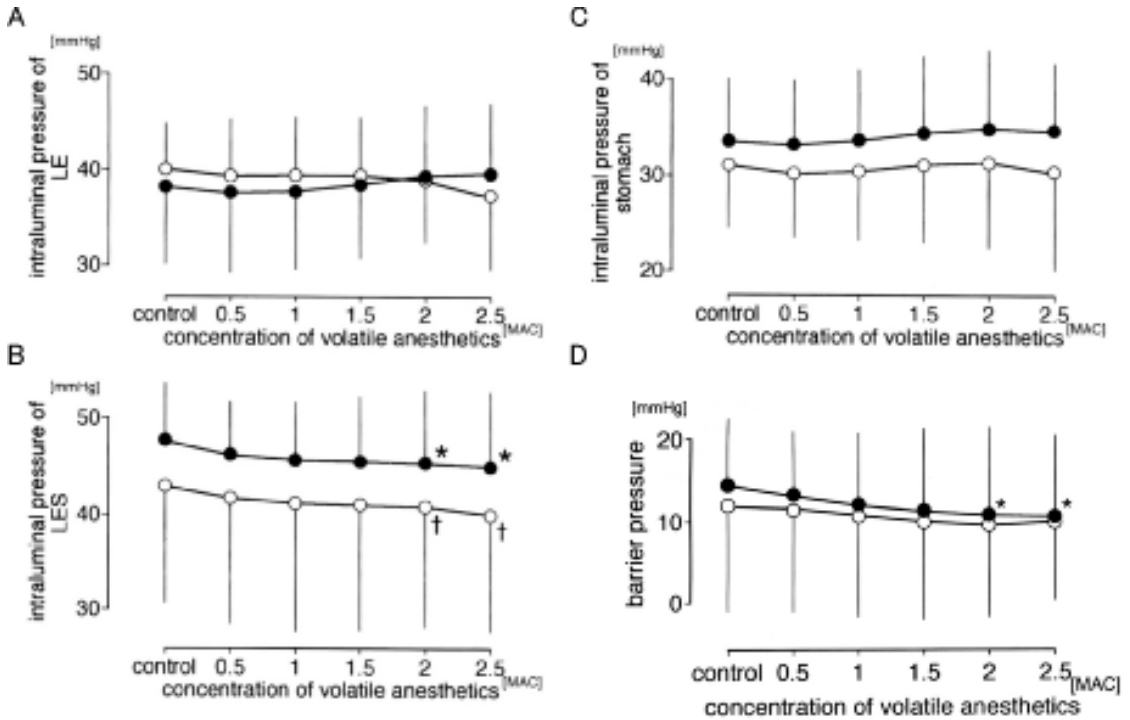


図 4 全身麻酔中の小児において、セボフルラン (open circles), エンフルラン (closed circles) 濃度を变化させたときの(A)下部食道, (B)LES, (C)胃の内圧変化および(D)胃 - 食道内圧較差 (barrier pressure) の変化。(Kohjitani A. et al. 1998⁵¹)から)

- mediator of non-adrenergic, non-cholinergic neuro-effector transmission. *Clin Exp Pharmacol Physiol* 19, 147-169, 1992
- 6) Hirsch, D. P., Holloway, R. H., Tytgat, G. N., Boeckstaens, G. E.: Involvement of nitric oxide in human transient lower esophageal sphincter relaxations and esophageal primary peristalsis. *Gastroenterology* 115, 1374-1380, 1998
 - 7) Bredt, D. S., Snyder, S. H.: Nitric oxide: a physiologic messenger molecule. *Annu Rev Biochem* 63, 175-195, 1994
 - 8) Shah, V., Lyford, G., Gores, G., Farrugia, G.: Nitric oxide in gastrointestinal health and disease. *Gastroenterology* 126, 903-913, 2004
 - 9) Tottrup, A., Knudsen, M. A., Gregersen, H.: The role of the L-arginine-nitric oxide pathway in relaxation of the opossum lower oesophageal sphincter. *Br J Pharmacol* 104, 113-116, 1991
 - 10) Tottrup, A., Svane, D., Forman, A.: Nitric oxide mediating NANC inhibition in opossum lower esophageal sphincter. *Am J Physiol* 260, G385-389, 1991
 - 11) Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., Freeman, B. A.: Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 87, 1620-1624, 1990
 - 12) Gryglewski, R. J., Palmer, R. M., Moncada, S.: Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 320, 454-456, 1986
 - 13) Rand, M. J., Li, C. G.: Nitric oxide as a neurotransmitter in peripheral nerves: nature of transmitter and mechanism of transmission. *Annu Rev Physiol* 57, 659-682, 1995
 - 14) Gibson, A., Brave, S. R., McFadzean, I., Tucker, J. F., Wayman, C.: The nitroergic transmitter of the anococcygeus --NO or not? *Arch Int Pharmacodyn Ther* 329, 39-51, 1995
 - 15) Martin, W., McAllister, K. H., Paisley, K.: NANC neurotransmission in the bovine retractor penis muscle is blocked by superoxide anion following inhibition of superoxide dismutase with diethyldithiocarbamate. *Neuropharmacology* 33, 1293-1301, 1994
 - 16) Thomas, R. M., Fang, S., Leichus, L. S., Oberley, L. W., Christensen, J., Murray, J. A., Ledlow, A., Conklin, J. L.: Antioxidant enzymes in intramural nerves of the opossum esophagus. *Am J Physiol* 270, G136-142, 1996
 - 17) Garthwaite, J., Charles, S. L., Chess-Williams, R.: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 336, 385-388, 1988
 - 18) Bredt, D. S., Snyder, S. H.: Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc Natl Acad Sci U S A* 86, 9030-9033, 1989
 - 19) Garthwaite, J., Garthwaite, G., Palmer, R. M., Moncada, S.: NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *Eur J Pharmacol* 172, 413-416, 1989
 - 20) Wood, P. L., Emmett, M. R., Rao, T. S., Cler, J., Mick, S., Iyengar, S.: Inhibition of nitric oxide synthase blocks N-methyl-D-aspartate-, quisqualate-, kainate-, harmaline-, and pentylentetrazole-dependent increases in cerebellar cyclic GMP in vivo. *J Neurochem* 55, 346-348, 1990
 - 21) East, S. J., Garthwaite, J.: NMDA receptor activation in rat hippocampus induces cyclic GMP formation through the L-arginine-nitric oxide pathway. *Neurosci Lett* 123, 17-19, 1991
 - 22) Gonzales, J. M., Loeb, A. L., Reichard, P. S., Irvine, S.: Ketamine inhibits glutamate-, N-methyl-D-aspartate-, and quisqualate-stimulated cGMP production in cultured cerebral neurons. *Anesthesiology* 82, 205-213, 1995
 - 23) Meldrum, B., Garthwaite, J.: Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol Sci* 11, 379-387, 1990
 - 24) Lipton, S. A., Rosenberg, P. A.: Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 330, 613-622, 1994
 - 25) Rothman, S., Olney, J.: Excitotoxicity and the NMDA receptor. *Trends Neurosci* 10, 299-302, 1987
 - 26) Hinoi, E., Takarada, T., Ueshima, T., Tsuchihashi, Y., Yoneda, Y.: Glutamate signaling in peripheral tissues. *Eur J Biochem* 271, 1-13, 2004
 - 27) Carlton, S. M.: Peripheral excitatory amino acids. *Curr Opin Pharmacol* 1, 52-56, 2001
 - 28) Aas, P., Tanso, R., Fonnum, F.: Stimulation of peripheral cholinergic nerves by glutamate indicates a new

- peripheral glutamate receptor. *Eur J Pharmacol* 164, 93-102, 1989
- 29) Rossetti, Z. L., Mameli, M., Vargiu, R., Fadda, F., Mancinelli, R.: Biphasic effects of NMDA on the motility of the rat portal vein. *Br J Pharmacol* 129, 156-162, 2000
 - 30) Liu, M. T., Rothstein, J. D., Gershon, M. D., Kirchgessner, A. L.: Glutamatergic enteric neurons. *J Neurosci* 17, 4764-4784, 1997
 - 31) Robertson, B. S., Satterfield, B. E., Said, S. I., Dey, R. D.: N-methyl-D-aspartate receptors are expressed by intrinsic neurons of rat larynx and esophagus. *Neurosci Lett* 244, 77-80, 1998
 - 32) Moroni, F., Luzzi, S., Franchi-Micheli, S., Zilletti, L.: The presence of N-methyl-D-aspartate-type receptors for glutamic acid in the guinea pig myenteric plexus. *Neurosci Lett* 68, 57-62, 1986
 - 33) Luzzi, S., Zilletti, L., Franchi-Micheli, S., Gori, A. M., Moroni, F.: Agonists, antagonists and modulators of excitatory amino acid receptors in the guinea-pig myenteric plexus. *Br J Pharmacol* 95, 1271-1277, 1988
 - 34) Wiley, J. W., Lu, Y. X., Owyang, C.: Evidence for a glutamatergic neural pathway in the myenteric plexus. *Am J Physiol* 261, G693-700, 1991
 - 35) Shannon, H. E., Sawyer, B. D.: Glutamate receptors of the N-methyl-D-aspartate subtype in the myenteric plexus of the guinea pig ileum. *J Pharmacol Exp Ther* 251, 518-523, 1989
 - 36) Sinsky, M., Donnerer, J.: Evidence for a neurotransmitter role of glutamate in guinea pig myenteric plexus neurons. *Neurosci Lett* 258, 109-112, 1998
 - 37) Cosentino, M., De Ponti, F., Marino, F., Giaroni, C., Leoni, O., Lecchini, S., Frigo, G.: N-methyl-D-aspartate receptors modulate neurotransmitter release and peristalsis in the guinea pig isolated colon. *Neurosci Lett* 183, 139-142, 1995
 - 38) Kohjitani, A., Shirakawa, J., Okada, S., Obara, H.: The relaxing effect of ketamine on isolated rabbit lower esophageal sphincter. *Anesth Analg* 84, 433-437, 1997
 - 39) Kohjitani, A., Miyawaki, T., Funahashi, M., Mitoh, Y., Matsuo, R., Shimada, M.: Intravenous anesthetics inhibit nonadrenergic noncholinergic lower esophageal sphincter relaxation via nitric oxide-cyclic guanosine monophosphate pathway modulation in rabbits. *Anesthesiology* 95, 176-183, 2001
 - 40) Kohjitani, A., Miyawaki, T., Funahashi, M., Higuchi, H., Matsuo, R., Shimada, M.: Ketamine and midazolam differentially inhibit nonadrenergic noncholinergic lower esophageal sphincter relaxation in rabbits: role of superoxide anion and nitric oxide synthase. *Anesthesiology* 98, 449-458, 2003
 - 41) Kemp, J., Foster, A., Wong, E.: Non-competitive antagonists of excitatory amino acid receptors. *Trends Neurosci* 10, 294-298, 1987
 - 42) Kohjitani, A., Funahashi, M., Miyawaki, T., Hanazaki, M., Matsuo, R., Shimada, M.: Peripheral N-methyl-D-aspartate receptors modulate nonadrenergic noncholinergic lower esophageal sphincter relaxation in rabbits. *Anesth Analg* 101, 1681-1688, 2005
 - 43) Kohjitani, A., Miyawaki, T., Funahashi, M., Mitoh, Y., Matsuo, R., Shimada, M.: Mexiletine inhibits nonadrenergic noncholinergic lower oesophageal sphincter relaxation in rabbits. *Eur J Pharmacol* 465, 145-151, 2003
 - 44) Lehmann, A., Branden, L.: Effects of antagonism of NMDA receptors on transient lower esophageal sphincter relaxations in the dog. *Eur J Pharmacol* 431, 253-258, 2001
 - 45) Hirsch, D. P., Tytgat, G. N., Boeckxstaens, G. E.: Is glutamate involved in transient lower esophageal sphincter relaxations? *Dig Dis Sci* 47, 661-666, 2002
 - 46) Baig, M. K., Wexner, S. D.: Postoperative ileus: a review. *Dis Colon Rectum* 47, 516-526, 2004
 - 47) Kalf, J. C., Schraut, W. H., Billiar, T. R., Simmons, R. L., Bauer, A. J.: Role of inducible nitric oxide synthase in postoperative intestinal smooth muscle dysfunction in rodents. *Gastroenterology* 118, 316-327, 2000
 - 48) Cardenas, A., Moro, M. A., Hurtado, O., Leza, J. C., Lorenzo, P., Castrillo, A., Bodelon, O. G., Bosca, L., Lizasoain, I.: Implication of glutamate in the expression of inducible nitric oxide synthase after oxygen and glucose deprivation in rat forebrain slices. *J Neurochem* 74, 2041-2048, 2000
 - 49) Ng, A., Smith, G.: Gastroesophageal reflux and aspiration of gastric contents in anesthetic practice. *Anesth Analg* 93, 494-513, 2001
 - 50) Grant, I. S., Nimmo, W. S., Clements, J. A.: Lack of

- effect of ketamine analgesia on gastric emptying in man. *Br J Anaesth* 53, 1321-1323, 1981
- 51) Paskins, J. R., Lawson, J. O., Clayden, G. S.: The effect of ketamine anesthesia on anorectal manometry. *J Pediatr Surg* 19, 289-291, 1984
- 52) Freye, E., Sundermann, S., Wilder-Smith, O. H.: No inhibition of gastro-intestinal propulsion after propofol- or propofol/ketamine-N₂O/O₂ anaesthesia. A comparison of gastro-caecal transit after isoflurane anaesthesia. *Acta Anaesthesiol Scand* 42, 664-669, 1998
- 53) Badrinath, S., Avramov, M. N., Shadrick, M., Witt, T. R., Ivankovich, A. D.: The use of a ketamine-propofol combination during monitored anesthesia care. *Anesth Analg* 90, 858-862, 2000
- 54) Kohjitani, A., Shirakawa, J., Satoh, E., Kagawa, T., Nakajima, M., Obara, H.: Effects of sevoflurane and enflurane on lower esophageal sphincter pressure and gastroesophageal pressure gradient in children. *J Anesth* 13, 1-7, 1999

平成18年度 鹿児島大学歯学部公開講座

「歯科治療時の偶発症（ヒヤリ・ハット）について考える」

日 時：平成19年3月4日（日）午後1時～午後6時

会 場：始良郡歯科医師会館・口腔保健センター・会議室（霧島市溝辺町）

後 援：始良郡歯科医師会，大口市歯科医師会，鹿児島県歯科医師会

プログラム

1. 鹿児島大学歯学部長 挨拶
西川殷維 歯学部長
2. 始良郡歯科医師会会長 挨拶
森原和久 会長
3. 歯科治療時の全身的偶発症について
梶山加綱 教授（歯科麻酔全身管理学）
4. 歯周疾患治療時の偶発症とその対策について
瀬戸口尚志 助教授（歯周病態制御学）
5. 抜歯時の偶発症，神経麻痺とその対策について
中村典史 教授（口腔顎顔面外科学）
6. 小児の歯科治療時の偶発症とその対策について
山崎要一 教授（口腔小児発達学）
7. 歯科臨床における感染予防対策について
杉原一正 教授（顎顔面疾患制御学）
8. 総合討論とまとめ
9. 大口市歯科医師会会長 挨拶
有川公仁 会長

実施報告

世話人 杉原 一正

本年度の歯学部公開講座は、鹿児島県歯科医師会、始良郡歯科医師会、大口市歯科医師会との共催のもと、始良郡歯科医師会館で歯科医師、歯科衛生士、歯科助手の方々の63名の出席を得て平成19年3月4日（日）に開催された。

歯科臨床においては、毎日の診療中にヒヤリとしたりハットしたりすることがしばしばある。もし、歯科治療中に偶発症が起こったら、どのように対処したらよいか。また、日常の歯科診療において偶発症を予防するには日頃からどのようなことに注意しておいたらよいかを中心に考えるのが本講座のテーマである。

梶山教授は、異常高血圧、神経性ショック、誤嚥誤飲事故、過換気症候群などの歯科治療時に発生しやすい全身の偶発症について解説した。瀬戸口尚志助教授は、歯肉退縮、歯間空隙の拡大、歯肉の擦過傷、知覚過敏などの歯周疾患治療時の偶発症とその予防と対策について解説した。中村典史教授は、下顎智歯抜歯、下顎孔伝達麻酔、下顎小白歯部の根管治療、インプラント時にみられる偶発症としての下歯槽神経麻痺や舌神経麻痺の予防とその治療法について解説した。山崎要一教授は、治療への非協力や体動による軟組織や硬組織の損傷、修復物や治療器具の落下による誤嚥・誤飲、局所麻酔後の下唇の咬傷などの小児の歯科治療時の偶発症とその対策について解説した。杉原は、最近の新興・再興感染症について概説するとともに歯科臨床における感染に気をつけなければならない血液媒介感染症の感染予防対策についてスタンダード・プレコーション（標準予防策）を中心に解説した。講演の最後には、受講者と演者との間で活発な質疑応答が行われた。

最後になりましたが、今回の公開講座の実施にあたって多大なご協力を賜りました森原和久始良郡歯科医師会長、有川公仁大口市歯科医師会会長、米重 成人始良郡歯科医師会理事に心より感謝申し上げます。また、本公開講座の準備、実施にご協力いただきました医歯学総合研究科等事務部山下憲一郎係長、野元健宏庶務係員、吉永章悟経理係員にも深謝申し上げます。



鹿児島大学歯学部発表論文 [2005年 SCI (または JCR) リスト雑誌で公表された業績]

1. Fujiwara N, Tabata MJ, Endoh M, Ishizeki K, Nawa T.: Insulin-like growth factor-I stimulates cell proliferation in the outer layer of Hertwig's epithelial root sheath and elongation of the tooth root in mouse molars in vitro. **Cell Tissue Res**, 320, 69-75, 2005.
2. Yoshikubo H, Suzuki N, Takemura K, Hosono M, Yashima S, Iwamuro S, Takagi Y, Tabata MJ, Hattori A.: Osteoblastic activity and estrogenic response in the regenerating scale of goldfish, a good model of osteogenesis. **Life Sci**, 76, 2699-2709, 2005.
3. Kamikawa-Miyado M, Ogi H, Ogino Y, Katoh H, Suzuki K, Uemura M, Kitoh J, Oda S, Yamada G.: The morphological and histological characters of the male external genitalia of the house musk shrew, *Suncus murinus*. **Zool Sci**, 22, 463-468, 2005.
4. Yamanaka A, Gunji H, Ishida H.: Curvature, length, and cross-sectional geometry of the femur and humerus in anthropoid primates. **Am J Phys Anthropol**, 127, 46-57, 2005.
5. Shida T, Abe S, Sakiyama K, Agematsu H, Mitarashi S, Tamatsu Y, Ide Y.: Superficial and deep layer muscle-fiber properties of the mouse masseter before and after weaning. **Arch Oral Biol**, 50, 65-71, 2005.
6. Maejima M, Abe S, Sakiyama K, Agematsu H, Hashimoto M, Tamatsu Y, Ide Y.: Changes in tongue muscle fiber properties of mouse before and after weaning. **Arch Oral Biol**, 50, 988-993, 2005.
7. Sakiyama K, Abe S, Tamatsu Y, Ide Y.: Effects of stretching stress on the muscle contraction proteins of skeletal muscle myoblasts. **Biomed Res**, 26, 61-68, 2005.
8. Terayama H, Nito M, Nakamura Y, Iimura A, Itoh M, Tamatsu Y, Shimada K.: Corrosion casts of convoluted testicular arteries in mice and rats. **Arch Androl**, 51, 471-480, 2005.
9. Harada S, Kanemaru N.: Developmental changes of the taste sensation depending on the maturation of the taste bud and its distribution in mammals. **Chem Senses**, 30 Suppl 1, i56-i57, 2005.
10. Miura H, Kato H, Kusakabe Y, Tagami M, Miura-Ohnuma J, Ookura T, Shindo Y, Y, Ninomiya Y, Hino A.: Shh signaling and regulatory gene expression in mouse taste buds. **Chem Senses**, 30 Suppl 1, i50-i51, 2005.
11. Kusakabe Y, Kim MR, Miura H, Shindo Y, Ninomiya Y, Hino A.: Regional expression patterns of t1r family in the mouse tongue. **Chem Senses**, 30 Suppl 1, i23-i24, 2005.
12. Kusakabe Y, Shindo Y, Kim MR, Miura H, Ninomiya Y, Hino A.: cDNA microarray screening for Taste-bud-specific genes. **Chem Senses**, 30 Suppl 1, i12-i13, 2005.
13. Miura H, Kato H, Kusakabe Y, Ninomiya Y, Hino A.: Temporal changes in NCAM immunoreactivity during taste cell differentiation and cell lineage relationships in taste buds. **Chem Senses**, 30, 367-375, 2005.
14. Yamasaki K, Harada S, Higuchi I, Osame M, Ito G.: Fatigue and damage to the masseter muscle by prolonged low-frequency stimulation in the rat. **Arch Oral Biol**, 50, 1005-1013, 2005.
15. Nagata E, Okayama H, Ito HO, Semba I, Inoue M, Oho T.: Experimental infective endocarditis induced by human supragingival dental plaque in rats. **Eur J Oral Sci**, 113, 499-504, 2005.
16. Kawatoko S, Sato T, Kawabata Y, Suenaga K, Suenaga S, Morita Y, Indo H, Kawano K, Iwashita Y, Majima HJ, Sugihara K, Semba I.: A trial for histopathological subclassification of papillary cystadenoma lymphomatosum by 99Tcm-pertechnetate in a patient with multiple bilateral lesions of the parotid glands. **Dentomaxillofac Radiol**, 34, 218-221, 2005.
17. Tanuma J, Hirano M, Hirayama Y, Semba I, Ogawa K, Shisa H, Hiai H, Kitano M.: Genetic predisposition to 4NQO-induced tongue carcinogenesis in the rat. **Med Princ Pract**, 14, 297-305, 2005.
18. Spinola M, Leoni VP, Tanuma J, Pettinicchio A, Frattini M, Signoroni S, Agresti R, Giovanazzi R, Pilotti S, Bertario L, Ravagnani F, Dragani TA.: FGFR4 Gly388Arg polymorphism and prognosis of breast and colorectal cancer. **Oncol Rep**, 14, 415-419, 2005.
19. Murayama T, Takegoshi M, Tanuma J, Eizuru Y.: Analysis of human cytomegalovirus VL144 variability in low-

- passage clinical isolates in Japan. **Intervirology**, 48, 201-206, 2005.
20. Suminami Y, Kishi F, Nawata S, Murakami A, Sakaguchi Y, Sueoka K, Numa F, Sugino N, Kato H.: Promoter analyses of SCC antigen genes. **Biochim Biophys Acta**, 1727, 208-212, 2005.
 21. Hirota T, Suzuki Y, Hasegawa K, Obara K, Matsuda A, Akahoshi M, Nakashima K, Cheng L, Takahashi N, Shimizu M, Doi S, Fujita K, Enomoto T, Ebisawa M, Yoshihara S, Nakamura Y, Kishi F, Shirakawa T, Tamari M.: Functional haplotypes of IL-12B are associated with childhood atopic asthma. **J Allergy Clin Immunol**, 116, 789-795, 2005.
 22. Sato T, Ishida T, Tanaka K, Ohnishi Y, Irifune M, Mimura T, Nishikawa T.: Ameliorative and exacerbating effects of [pGlu4, Cyt6] AVP (4-9) on impairment of step-through passive avoidance task performance by group metabotropic glutamate receptor-related drugs in mice. **J Pharmacol Sci**, 97, 437-442, 2005.
 23. Sato T, Ishida T, Tanaka K, Chowdhury N, Irifune M, Hirate K, Mimura T, Nishikawa T.: Glutamate antagonists attenuate the action of NC-1900, a vasopressin fragment analog, on passive avoidance task performance in mice. **Peptides**, 26, 893-897, 2005.
 24. Ban S, Kono H, Iwaya Y, Yuda A, Izumi Y.: Effect of concentrated sulfuric acid-etching on apatite-forming ability of alkaline-treated titanium. **Key Engineering Materials**, 284-286, 525-530, 2005.
 25. Watanabe T, Ban S, Itoh T, Tsuruta S, Kawai T, Nakamura H.: Physical properties of composite membrane containing apatite and poly-lactic acid/poly-glycolic acid copolymer in vitro. **Key Engineering Materials**, 284-286, 749-752, 2005.
 26. Itoh T, Ban S, Watanabe T, Tsuruta S, Kawai T, Nakamura H.: Bioactivity of the two kinds of biodegradable composite membrane containing oriented needle-like apatite. **Key Engineering Materials**, 284-286, 811-814, 2005.
 27. Kanie T, Arikawa H, Fujii K, Ban S.: Deformation and flexural properties of denture base polymer reinforced with glass fiber sheet. **Dent Mater J**, 24, 297-303, 2005.
 28. Kanie T, Kadokawa A, Arikawa H, Fujii K, Ban S.: Mechanical properties of an experimental soft lining material based on urethane oligomer. **Dent Mater J**, 24, 433-439, 2005.
 29. Yuda A, Ban S, Izumi Y.: Biocompatibility of apatite-coated titanium mesh prepared by hydrothermal-electrochemical method. **Dent Mater J**, 24, 588-595, 2005.
 30. Arikawa H, Kanie T, Fujii K, Ban S.: Effect of radiation light characteristics on surface hardness of paint-on resin for shade modification. **Dent Mater J**, 24, 636-641, 2005.
 31. Fujii K, Miami H, Arikawa H, Kanie T, Ban S, Inoue M.: Mechanical properties and bond strength of silicone-based resilient denture liners. **Dent Mater J**, 24, 667-675, 2005.
 32. Miwa Y, Ito H-O, Inoue M, Oho T.: Induction of immune response to *Streptococcus pneumoniae* by administration of oral viridans streptococci via phosphorylcholine determinant. **FEMS Immunol Med Microbiol**, 43, 441-448, 2005.
 33. Yamaguchi T, Miwa Y, Eizuru Y.: Candidate Human Papillomavirus (HPV) Type 27b: nucleotide sequence and heterogeneity with HPV 27. **J Med Virol**, 77, 113-115, 2005.
 34. Okayama H, Nagata E, Ito H-O, Oho T, Inoue M.: Experimental abscess formation caused by human dental plaque. **Microbiol Immunol**, 49, 399-405, 2005.
 35. Yamaguchi T, Sharma P, Athanasios M, Kumar A, Yamada S, Kuehn M.: Mutation of SENP1/SuPr-2 reveals an essential role for desumoylation in mouse development. **Mol Cell Biol**, 25, 5171-5182, 2005.
 36. Tokuda M, Miyamoto R, Sakuta T, Nagaoka S, Torii M.: Substance P activates p38 mitogen-activated protein kinase to promote IL-6 induction in human dental pulp fibroblasts. **Connect Tissue Res**, 46, 153-158, 2005.
 37. Miyamoto R, Tokuda M, Sakuta T, Nagaoka S, Torii M.: Expression and characterization of vanilloid receptor subtype 1 in human dental pulp cell cultures. **J Endod**, 31, 652-658, 2005.
 38. Tancharoen S, Sarker KP, Imamura T, Biswas KK, Matsushita K, Tatsuyama S, Travis J, Potempa J, Torii M, Maruyama I.: Neuropeptide release from dental pulp cells by RgpB via protease-activated receptor-2 signaling.

J Immunol, 174, 5796-5804, 2005.

39. Aoki D, Ueno S, Kubo F, Oyama T, Sakuta T, Matsushita K, Maruyama I, Aikou T.: Roxithromycin inhibits angiogenesis of human hepatoma cells in vivo by suppressing VEGF production. **Anticancer Res**, 25, 133-138, 2005.
40. Ueno S, Aoki D, Kubo F, Hiwatashi K, Matsushita K, Oyama T, Maruyama I, Aikou T.: Roxithromycin inhibits constitutive activation of nuclear factor B by diminishing oxidative stress in a rat model of hepatocellular carcinoma. **Clin Cancer Res**, 11, 5645-5650, 2005.
41. Matsuyama T, Kawai T, Izumi Y, Taubman MA.: Expression of Major Histocompatibility Complex Class II and CD80 by Gingival Epithelial Cells Induces Activation of CD4+ T Cells in Response to Bacterial Challenge. **Infect Immun**, 73, 1044-1051, 2005.
42. Nitta H, Wara-aswapati N, Lertsirivorakul J, Nakamura T, Yamamoto M, Izumi Y, Nakamura T, Ishikawa I.: A Novel Mutation of the Cathepsin C Gene in a Thai Family with Papillon-Lefevre Syndrome. **J Periodontol**, 76, 492-496, 2005.
43. Kosaka S, Kajihara H, Kurashige H, Tanaka T.: Effect of resin coating as a means of preventing marginal leakage beneath full cast crowns. **Dent Mater J**, 24, 117-122, 2005.
44. Minami H, Suzuki S, Ohashi H, Minesaki Y, Tanaka T.: In vitro evaluation of the bonding of auto-polymerizing soft denture liner to cobalt-chromium alloy. **J Oral Rehabil**, 32, 454-460, 2005.
45. Kajihara H, Suzuki S, Minesaki Y, Kurashige H, Tanaka T.: Effect of filler loading on resin cement bonding to silanized buildup composites. **Am J Dent**, 18, 109-112, 2005.
46. Suzuki S, Minami H.: Evaluation of toothbrush and generalized wear of luting materials. **Am J Dent**, 18, 311-317, 2005.
47. Miyawaki S, Araki Y, Tanimoto Y, Katayama A, Fujii A, Imai M, Takano-Yamamoto T.: Occlusal force and condylar motion in patients with anterior open bite. **J Dent Res**, 84, 133-137, 2005.
48. Deguchi T, Honjo T, Fukunaga T, Miyawaki S, Roberts WE, Takano-Yamamoto T.: Clinical assessment of orthodontic outcomes using Peer Assessment Rating, Discrepancy Index, Objecting Grading System and Comprehensive Clinical Assessment. **Am J Orthod Dentofac Orthop**, 127, 434-443, 2005.
49. Kuratani T, Miyawaki S, Murakami T, Takano-Yamamoto T.: Early orthodontic treatment and long-term observation in a patient with Morquio syndrome. **Angle Orthod**, 75, 881-887, 2005.
50. Tanimoto Y, Miyawaki S, Imai M, Takeda R, Takano-Yamamoto T.: Orthodontic treatment of a patient with an impacted maxillary second premolar and odontogenic keratocyst in the maxillary sinus. **Angle Orthod**, 75, 1077-1083, 2005.
51. Ishitani N, Masumoto Y, Yoshihara T, Yamasaki Y.: Changes in electroencephalographic activities following pressure stimulation in humans. **Psychiatry Clin Neurosci**, 59, 644-651, 2005.
52. Yen HC, Chang HM, Majima HJ, Chen FY, Li SH.: Levels of reactive oxygen species and primary antioxidant enzymes in WI38 versus transformed WI38 cells following bleomycin treatment. **Free Radic Biol Med**, 38, 950-959, 2005.
53. Kakinuma S, Nishimura M, Kubo A, Nagai JY, Amasaki Y, Majima HJ, Sado T, Shimada Y.: Frequent retention of heterozygosity for point mutations in p53 and Ikaros in N-ethyl-N-nitrosourea-induced mouse thymic lymphomas. **Mutat Res**, 572(1-2), 132-141, 2005.
54. Yen HC, Tang YC, Chen FY, Chen SW, Majima HJ.: Enhancement of Cisplatin-induced apoptosis and caspase 3 activation by depletion of mitochondrial DNA in a human osteosarcoma cell line. **Ann N Y Acad Sci**, 1042, 516-522, 2005.
55. Ijiri K, Tsuruga H, Sakakima H, Tomita K, Taniguchi N, Shimoonoda K, Komiya S, Goldring MB, Majima HJ, Matsuyama T.: Increased expression of humanin peptide in diffuse-type pigmented villonodular synovitis: implication of its mitochondrial abnormality. **Ann Rheum Dis**, 64, 816-823, 2005.

56. Sato T, Indo H, Kawabata Y, Kobayashi T, Suenaga S, Iwashita Y, Nitta T, Sugihara K, Majima HJ.: Thallium-201 chloride (Tl-201) accumulation and Na⁺/K⁺-ATPase expression in tumours of the head and neck. **Dentomaxillofac Radiol**, 34, 212-217, 2005.
57. Sato T, Kawabata Y, Nitta T, Saigo Y, Iwashita Y, Suenaga S, Hamahira S, Indo H, Morita Y, Majima HJ, Sugihara K.: Expression of permeability-glycoprotein (P-gp) and uptake of technetium-99m-hexakis-2-methoxyisobutyl-isonitrile (99Tcm-MIBI) in malignant tumour of the head and neck. **Dentomaxillofac Radiol**, 34, 274-278, 2005.
58. Sato T, Kawabata Y, Kobayashi Y, Suenaga S, Indo H, Kawano K, Iwashita Y, Morita Y, Majima HJ.: Scintigraphy for interpretation of malignant tumours of the head and neck: comparison of technetium-99m-hexakis-2-methoxyisobutylisonitrile (Tc-MIBI) and thallium-201-chloride (Tl-201). **Dentomaxillofac Radiol**, 34, 268-273, 2005.
59. Hamada T, Goto M, Tsutsumida H, Nomoto M, Higashi M, Sugai T, Nakamura S, Yonezawa S.: Mapping of the methylation pattern of the MUC2 promoter in pancreatic cancer cell lines using bisulfite genomic sequencing. **Cancer Letters**, 227, 175-184, 2005.
60. Tabata M, Shimoda T, Sugihara K, Ogomi D, Ohgudhi H, Asashi M.: Appetite formed on/in agarose gel as a bone-grafting materials in the treatment of periodontal infrabony defect. **Mater Res Part, Appl Biomater**, 75B, 378-386, 2005.
61. Goto M, Shibahara H, Tamada S, Hamada T, Oda K, Nagino M, Nagasaka T, Imai K, Nimura Y, Yonezawa S.: Aberrant expression on pyloric gland-type mucin-producing bile duct carcinoma: A clear difference between the core peptide and the carbohydrate moiety. **Pathol International**, 55, 464-470, 2005.
62. Saitou M, Goto M, Hironouchi M, Tamada S, Nagata K, Hamada T, Osako M, Takao S, Surinder K Batra, Aikou T, Yonezawa S.: MUC4 expression in novel prognostic factor in patients with invasive ductal carcinoma of the pancreas. **J Clin Pathol**, 58, 845-852, 2005.

伊藤学而名誉教授への御礼

伊藤学而名誉教授から鹿児島大学歯学部へ多額のご寄付を頂戴いたしました。ここに報告させて頂きま
すとともに厚く御礼申し上げます。 歯学部長 西川殷維

編 集 後 記

鹿児島大学歯学部紀要第27巻をお届けいたします。今回は、平成19年3月31日付で鹿児島大学大学院医歯学総合研究科生体機能制御学講座歯科応用薬理学分野（旧：歯学部歯科薬理学講座）を定年退職されます西川殷維先生と新しく顎顔面機能再建学講座歯科麻酔全身管理学分野（旧：歯科麻酔科）の助教授に着任されました糺谷 淳先生に総説論文を書いていただきました。ご多忙の中、執筆いただきました両先生に心より御礼申し上げます。

西川殷維教授は歯学部にて27年間もの長い間勤務され歯学部における教育と研究に従事され、平成15年4月から平成19年3月まで2期4年間にわたり歯学部長を務められ、歯学部の管理運営と発展に尽力されました。また、顎顔面機能再建学講座歯周病態制御学分野（旧：歯科保存学第2講座）の和泉雄一教授が平成19年4月1日付で東京医科歯科大学へ転出され、発生発達成育学講座分子遺伝学分野（旧：口腔細菌学講座）の岸 文雄教授が平成19年4月1日付で川崎医科大学へ転出されます。

昨年度の歯学部公開講座は、始良郡歯科医師会館で平成19年3月4日（日）に開催され63名の受講者を得て盛会裡に終了しましたので、その報告もさせていただきます。

それから、毎号掲載しております旧歯学部教員の業績（SCIまたはJCRリスト雑誌掲載論文）も昨年度と同じくらい掲載されています。国立大学法人鹿児島大学となり研究費等の厳しい状況が続いていますが、今後も旧歯学部教職員は益々多くの研究業績や業務実績があげられるようお互いがんばっていききたいものです。

最後に本誌の発行にご協力いただきました医歯学総合研究科等事務部をはじめ関係各位に感謝申し上げます。

（編集委員 杉原一正）

平成 19 年 3 月 15 日 印刷

平成 19 年 3 月 26 日 発行

発行所

鹿児島大学歯学部 代表 西川 殷維
鹿児島市桜ヶ丘八丁目35 - 1

印刷所

斯 文 堂 株 式 会 社
鹿児島市南栄2 - 12 - 6
電話番号 099 - 268 - 8211