Methamphetamine-withdrawal Stress Activates PACAP-DBI Pathway in Rat Salivary Gland, Resulting in Inhibition of Salivary Secretion

Migiwa OKUBO[*1, 2], Masanobu YOSHIKAWA[*2, 3], Takashi SHINOMIYA[*1, 2] and
Mitsuru KAWAGUCHI[*2]

[*1]Oral Health Science Center hrc8, [*2]Department of Pharmacology, Tokyo Dental College
[*3]Department of Clinical Pharmacology, Tokai University School of Medicine

The purpose of this study was to investigate activation of inhibitory regulation pathways by methamphetamine (METH)-withdrawal stress in rat salivary gland. Our previous study showed that METH-withdrawal stress activated steroid biosynthesis and that pregnenolone produced during the early stage of this process inhibited salivary secretion. However, how this type of stress inhibits salivary secretion and the activation pathway of steroid biosynthesis in salivary gland remain to be clarified. In the present study, using an *in vivo* cannulation method, METH-withdrawal stress decreased salivary secretion and increased expression of diazepam-binding inhibitor (DBI), an endogenous peripheral-type benzodiazepine receptor (PBR) agonist; Western blot and RT-PCR also showed increased expression of DBI mRNA in parotid, submandibular, and sublingual gland. In addition, METH-withdrawal stress also elicited an increase in pituitary adenylate cyclase–activating polypeptide (PACAP) and PBR mRNA, which is associated with DBI activity. These results suggest that METH-withdrawal stress activates a PACAP-DBI pathway in salivary gland, enhancing steroid genesis and inhibiting secretion.

Key words: ʻSalivary secretionʼ ʻMethamphetamine withdrawal stressʼ ʻDiazepam binding inhibitorʼ ʻPituitary adenylate cyclase–activating polypeptideʼ ʻpregnenoloneʼ

INTRODUCTION

In earlier studies, we showed that gamma-aminobutyric acid A receptor (GABA$_A$-R) and benzodiazepine receptors mediated inhibitory mechanisms in salivary gland [6–8, 15, 16]. We also demonstrated that stress activated steroid biosynthesis in salivary gland using a rat model of stress induced by methamphetamine (METH)-withdrawal and that pregnenolone (PRG) produced during the early stage of this process inhibited salivary secretion through the GABA$_A$-receptor using rat submandibular gland perfusion method [13]. However, how this type of stress inhibits salivary secretion and the activation pathway of steroid biosynthesis in salivary gland remain to be clarified.

Diazepam-binding inhibitor (DBI) is an endogenous agonist of benzodiazepine receptor [17]. Binding of DBI to peripheral type benzodiazepine receptor (PBR) in mitochondria produced neurosteroids such as PRG, which can modulate GABA$_A$ receptor activity [11]. Several studies showed that repetitive administration of morphine [3, 9], ethanol [4], nicotine [5], or METH [20] increased DBI mRNA in the central nervous system (CNS). Expression of DBI is regulated at the transcriptional level through pituitary adenylate cyclase–activating polypeptide (PACAP) [12, 20]. Previously, we reported that these pathways also existed in salivary gland and that they correlated with inhibition of salivary secretion [21]. Stress induced by METH withdrawal may activate steroid biosynthesis via a PACAP-DBI pathway in salivary gland, resulting in inhibition

of salivary secretion. The purpose of the present study was to investigate inhibition of salivary secretion using an *in vivo* cannulation method. In addition, the effect of METH-withdrawal stress on expression of DBI and on PACAP or PBR, substances related to DBI activity, was also examined.

MATERIALS AND METHODS

Animals

Male Wistar strain rats weighing 200–250 g each were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were kept for one week prior to the experiments (constant room temperature, 23 ± 2℃ ; humidity, 55% ± 5%; 12-hr light/dark cycle, with lighting from 6:00 am to 6:00 pm; maintained on commercial laboratory chow and tap water). The animals were treated in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of Tokyo Dental College, and were approved by the Animal Experimentation Committee of Tokyo Dental College.

Materials

Methamphetamine hydrochloride was supplied by Dainippon Sumitomo Pharma, Osaka, Japan. Pilocarpine, carbamylcholine chloride (CCh), and steroids were purchased from Sigma St. Louis, MO, USA. All other reagents used were of the highest grade commercially available.

Preparation of METH withdrawal-stress rat

Induction of stress by METH withdrawal was

Migiwa OKUBO, Department of Pharmacology, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
Tel: +81-43-270-3774   Fax: +81-43-270-3776   E-mail: okubo@tdc.ac.jp

performed as described in our previous study [13]. Briefly, the rats received intraperitoneal injection of METH (1 mg/kg) twice a day for 2 weeks. One week after terminating administration, salivary secretion was investigated and parotid gland (PG), submandibular gland (SMG), sublingual gland (SLG), and cerebral cortex (Cx) harvested for quantitative RT-PCR. The control group received physiological saline instead of METH.

Collection of saliva from PG, SMG, and SLG by in vivo cannulation method

Saliva was collected by an *in vivo* cannulation method as described previously [22]. Briefly, the rats were secured in a supine position, anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and a tracheal tube inserted to support respiration. The tapered end of a capillary cannula was inserted into the parotid duct, sublingual papillae, and sublingual openings to obtain saliva from PG, SMG, SLG, respectively, while the other end was placed inside a 0.5-ml microtube to collect secreted saliva. Saliva was collected every 15 min over 1 hr by stimulation with pilocarpine (1.0 mg/kg, i.p.).

Extraction of total RNA and real time quantitative RT-PCR

The salivary glands and brain were removed to allow PG, SMG, SLG, and Cx to be harvested for gene analysis by RT-PCR. Total RNA was treated with DNase 1 and first-strand cDNA synthesized using Oligo(dT)$_{12-18}$ primers and Superscript™ III RNase H-reverse transcriptase. Gene expression of DBI, PACAP, and PBR was determined using the beta actin (accession number NM_031144) (Left: 5'-GGAGATTACTGCCC TGGCTCCTA-3', Right: 5'-GACTCATCGTACTCCTGCTTGCTG-3') gene as an internal control and primers specific for DBI mRNA (accession number NM_031853) (Left: 5'-ACGCTCTGGAACTTGATTGC-3'; Right: 5'-CAGTTGGCTGAGTCTTGAG G-3'), PACAP mRNA (accession number NM_016989) (Left: 5'- TGTCCGCCAGGAAGTACC-3'; Right: 5'-CCGAGTGGCGTTTGGTAA-3'), PBR mRNA (NM_012515) (Left: 5'-ACACTGGTCAGCTGGCTCTGAA-3'; Right: 5'-CAGGCCAGGTAAGGATACAGCAA-3') cDNA was amplified by real-time quantitative PCR using the DyNAmo SYBER green qPCR Kit (Finnzymes; Espoo, Finland) on the DNA Engine Opticon 2 System (Bio-Rad Laboratories; Hercules, CA), running 48 cycles of the following protocol: 15 min predenaturation at 95℃, 10 sec denaturation at 94℃, 20 sec annealing at 60℃ for beta actin or 58.6℃ for DBI, PACAP, and PBR, followed by a 30-sec extension at 72℃. The PCR products were quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), which utilizes chip-based nucleic acid separation technology. Ten-fold serial dilutions of cDNA were used to construct a standard curve from 10^6 to 10^1 copies. The standard curve was generated from threshold values, and linear regression analysis provided a straight-line plot, which indicated our system was able to accurately quantify mRNA. Melting curve analysis

also revealed that DBI, PACAP, PBR, or Beta-Actin-specific primer pair amplified a single predominant product.

Western blot analysis for DBI

For Western blot analysis of DBI, Cx, PG, SMG, or SLG was homogenized in RIPA Buffer (150 mM NaCl, 1% NP-40, 0.5% deoxychorate-Na, 0.1% SDS and 50 mM Tris-HCl, pH 8.0) containing complete mini-protease inhibitor cocktail tablets (Roche Diagnostics, Mannheim, Germany) and incubated for 30 min on ice. After centrifuging at $14,000 \times g$ for 15 min at 4℃, the resulting supernatants were boiled in SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer for 10 min at 70℃. The proteins were subjected to SDS-PAGE using 4–12% NuPage Bis-Tris gels with MES Running Buffer (Invitrogen, San Diego, CA USA). Proteins were electrically transferred onto poly-vinylidene fluoride membranes (Immobilon P, Nippon Millipore; Tokyo, Japan) at 20 V for 1.5 hr. Membranes were blocked for 1 h at room temperature with 0.5% bovine serum albumin in Tris-buffered saline (TBS). The membrane was washed in TBS containing 0.05% Tween-20 (T-TBS) then incubated overnight at 4℃ in primary antibody consisting of anti-DBI (1:10000; sc-30190, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-beta-actin (1:5000; A1978, Sigma Aldrich Japan), followed by horseradish peroxidase–linked secondary antibody. Signal detection was carried out using Pierce Western Blotting Substrate Plus (Thermo Scientific, Tokyo Japan). The western blot band images were captured using a CCD camera system, Cool Saver (ATTO, Tokyo Japan). The specific bands were then analyzed for density using a CS Analyzer (ATTO). Beta-Actin protein expression was quantified to normalize the amount of DBI protein in each sample.

Collection of saliva by rat submandibular gland perfusion method

Saliva was collected by the rat SMG perfusion method as described previously [13]. The submandibular artery was isolated and a 29-G needle with a round tip inserted through a small incision. The artery was then perfused with Hank's balanced salt solution containing 1.27 mM CaCl$_2$, 0.81 mM MgSO$_4$ and 30 mM HEPES, pH 7.4 (HBSS-H) at 0.5 ml/min using the Perista mini-pump SJ-1220 (ATTO, Tokyo Japan). Carbamylcholine chloride (CCh), a muscarinic agonist and salivary stimulant, was administered over a 5-min period every 30 min during perfusion. Pretreatment with steroids was carried out 5 min before CCh stimulation. To collect saliva, the capillary end of a polyethylene tube (IMAMURA, Tokyo Japan) was inserted 3 mm into the submandibular duct toward the glandular body. Saliva was collected into microtubes and net weight determined. To correct for individual differences, these data were expressed as a percentage of secretion induced by CCh alone in each animal.

Statistical analysis

The data are expressed as the mean ± S.E.M. Statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by the
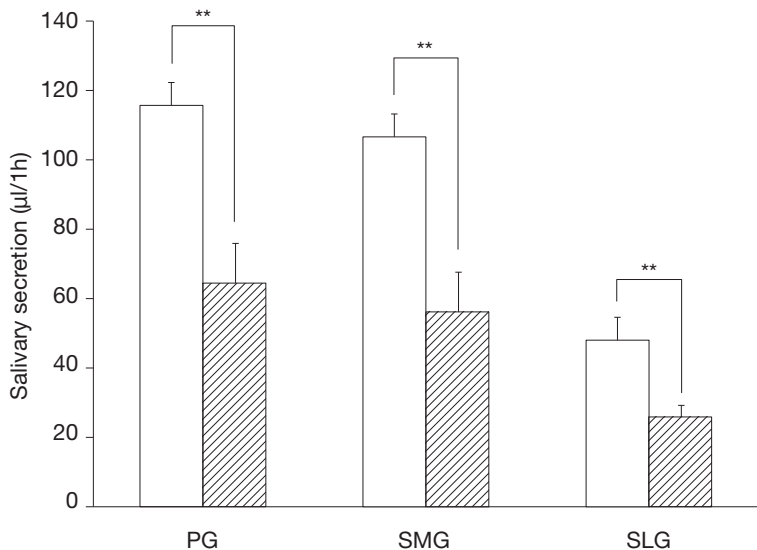
Fig. 1 *In vivo* inhibitory effect of methamphetamine (METH)-withdrawal stress on pilocarpine-induced salivary secretion in parotid gland (PG), submandibular gland (SMG), and sublingual gland (SLG) in experimental (striped columns) and control groups (white columns). Results represent mean with S.E.M. of 4 or 5 experiments. ** Significantly different from values in control group treated with saline at P < 0.01.

Dunnett's post-hoc test or the Mann-Whitney test. A value of P < 0.05 was considered to be statistically significant.

Disclosure statement

The authors declare that they had no conflicts of interest in regard to this study.

RESULTS

Inhibitory effect of methamphetamine (METH)-withdrawal stress on salivary secretion using in vivo cannulation method

Pilocarpine (1.0 mg/kg)-induced salivary secretion from PG, SMG, and SLG was 115.4 ± 6.7 μl/1 hr, 106.0 ± 8.6 μl/1 hr, and 47.6 ± 6.3 μl/1 hr, respectively (Fig.1). Stress induced by METH withdrawal resulted in a significant reduction in salivary secretion from PG, SMG, and SLG to 63.6 ± 12.1 μl/1 hr, 56.5 ± 10.7 μl/1 hr and 25.8 ± 3.2 μl/1 hr, respectively. Methamphetamine withdrawal stress induced an approximately 45% decrease in secretion in all types of salivary gland tested. No difference was observed in level of inhibition between each type of gland.

Effect of METH-withdrawal stress on expression of DBI, PACAP, and PBR mRNA in salivary glands and Cerebral cortex using real time quantitative RT-PCR

DBI, PACAP, and PBR mRNA expression in rat salivary gland and Cx was determined by real time quantitative RT-PCR (Fig.2). DBI, PACAP, and PBR mRNA were expressed in Cx PG, SMG, and SLG, with the level in each type of tissue showing a significant increase by METH-withdrawal stress. DBI mRNA levels increased to 1.7-, 2.0-, 1.9-, and 2.0-fold, PACAP mRNA levels to 2.0-, 7.0-, 4.0-, and 3.9-fold, PBR mRNA levels to 2.4-, 4.0-, 2.8-, and 2.2-fold of those in the control group in Cx, PG, SMG, and SLG, respectively, by METH-withdrawal stress.

Effect of METH-withdrawal stress on peptide levels of DBI in salivary glands and cerebral cortex using western blot analysis

Expression of DBI and PACAP protein was assessed by Western blot analysis. Bands indicating DBI (10 kD) and β-actin (42 kD) in SMG, SLG, PG, and Cx in METH-withdrawal stress and control rats are shown in Fig.3.a. Semiquantitative analysis was performed according to density of DBI bands and normalized to the density of the β-actin bands. The relative amounts of DBI protein in Cx, and PG, SMG, and SLG were 0.65, 0.67, 0.73, and 0.84, respectively (Fig.3.b). Following METH-withdrawal stress, production of DBI protein in Cx, and PG, SMG, and SLG significantly increased to 1.5-1.9-fold that in the control group: Cx, 1.5-fold increase; PG, 1.6-fold increase; SMG, 1.5-fold increase; and SLG, 1.9-fold increase.

Effects of steroids on CCh-induced salivary secretion in rat submandibular gland perfusion system

We demonstrated the inhibitory effects of various steroids on salivary secretion, using a rat submandibular gland perfusion method. Allopregnanolone (AP), testosterone (TES), hydrocortisone (HC), progesterone (PRO), estradiol (EST) and PRG showed inhibition of CCh-induced salivary secretion with an inhibition-ratio of 72.4%, 73.5%, 67.5%, 65.5%, 73.8%, and 68.8%, respectively (Fig.4).

DISCUSSION

Clinical research has shown that chronic METH abuse can result in withdrawal symptoms such as anxiety and induce xerostomia [2, 19]. In this study, METH-withdrawal stress significantly decreased pilocarpine-induced salivary secretion in rat PG, SMG, and SLG (Fig.1). In our previous study, METH-withdrawal stress activated production of CYP11A1, a key enzyme in early-stage steroid biosynthesis, in PG, SMG, and SLG [13]. Pregnenolone produced by CYP11A1 inhibited salivary secretion in an in situ rat submandibular perfusion model [13] (Fig.4). In addition, PRG enhanced the inhibitory effects of muscimol, a GABA$_A$-R agonist, on salivary secretion. These inhibitory effects were blocked by bicuculline, a GABA$_A$-R antagonist [13]. These results suggested that stress-produced steroid hormones acted as autocrines, inhibiting salivary secretion by enhancing GABA$_A$-R regulation in salivary gland.

Production of PRG as a neurosteroid was promoted

a)

b)

c)
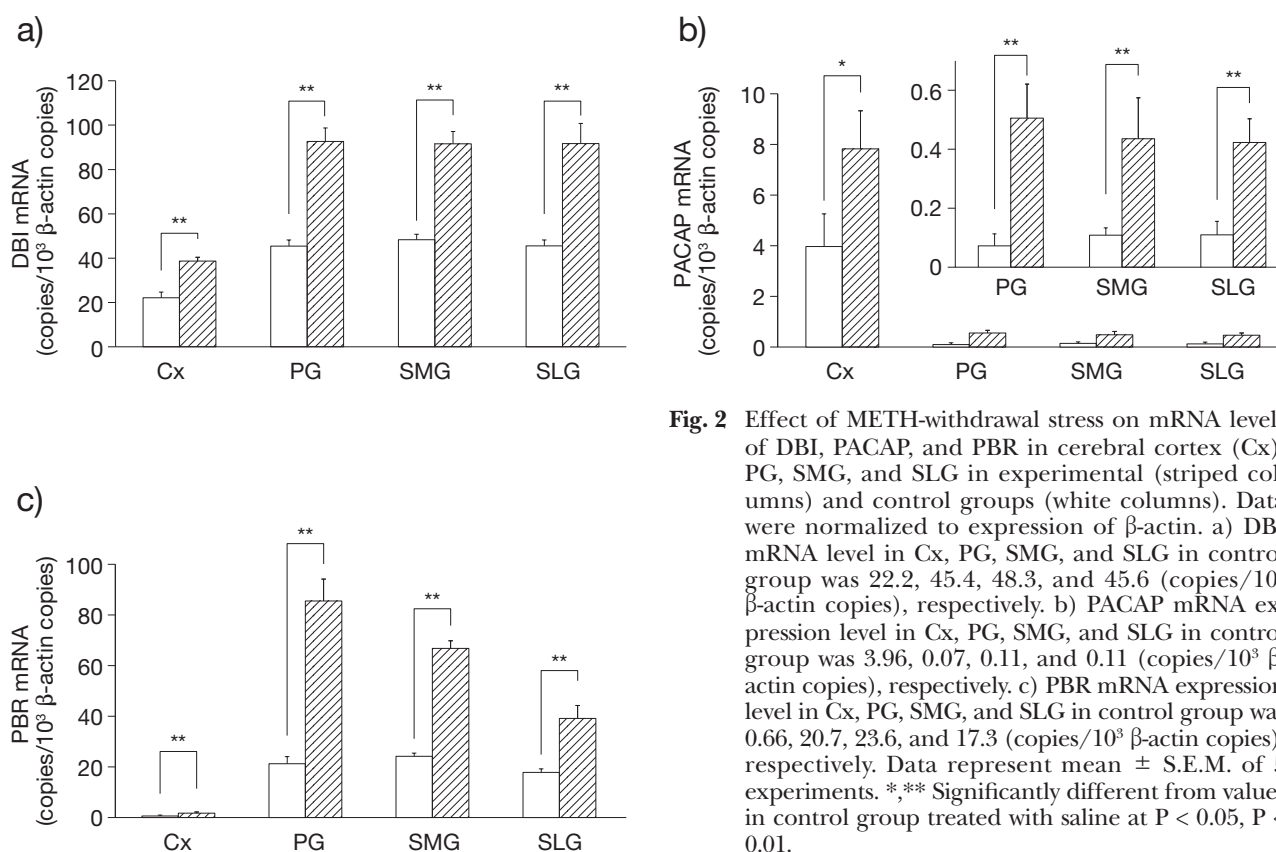
Fig. 2 Effect of METH-withdrawal stress on mRNA levels of DBI, PACAP, and PBR in cerebral cortex (Cx), PG, SMG, and SLG in experimental (striped columns) and control groups (white columns). Data were normalized to expression of β-actin. a) DBI mRNA level in Cx, PG, SMG, and SLG in control group was 22.2, 45.4, 48.3, and 45.6 (copies/10^3 β-actin copies), respectively. b) PACAP mRNA expression level in Cx, PG, SMG, and SLG in control group was 3.96, 0.07, 0.11, and 0.11 (copies/10^3 β-actin copies), respectively. c) PBR mRNA expression level in Cx, PG, SMG, and SLG in control group was 0.66, 20.7, 23.6, and 17.3 (copies/10^3 β-actin copies), respectively. Data represent mean $\pm$ S.E.M. of 5 experiments. *,** Significantly different from values in control group treated with saline at $P < 0.05$, $P < 0.01$.

by DBI via mitochondrial PBR in CNS [5]. In addition, DBI expression was regulated by PACAP through the activation of PACAP type I receptor (PAC1-R) [12, 20]. Recently, we showed that repetitive administration of diazepam inhibited salivary secretion and enhanced expression of DBI, PBR, and PACAP mRNA [21]. The protein level of DBI was consistent with its mRNA level in salivary gland. These results suggest that DBI production may result in an increase in the suppressive effect of diazepam on salivary secretion. In the present study, an increase was observed in DBI, PBR, and PACAP mRNA by METH-withdrawal stress (Fig.2). Levels of DBI protein also showed an increase with METH-withdrawal stress (Fig.3). We believe that this marked change in PACAP mRNA levels would have been sufficient to drive DBI production. However, the PACAP mRNA was significantly lower in each type of salivary gland than in Cx, indicating that some other pathway is also involved in production of DBI. METH-withdrawal stress was reported to enhance production of DBI by activation of peroxisome proliferator-activated receptor-γ(PPAR-γ) in CNS [18,19]. In addition, TNFα activated expression of PBR [18]. METH-withdrawal stress may enhance DBI activity via multiple pathways in salivary glands.

It is known that DBI acts not only as a PBR agonist, but also as an inverse agonist of central-type benzodiazepine receptor (CBR) (Fig.5). Increased DBI by METH-withdrawal stress was suggested to inhibit regulation of the CBR-GABA$_A$-R complex in CNS and induce anxiety [20]. If this theory also holds true for salivary gland, DBI would increase salivary secretion via GABA$_A$-R. However, our data show that salivary secretion decreased by METH-withdrawal stress or

repetitive administration of diazepam, which increases DBI. This means that DBI has a more potent effect on PBR than CBR in salivary gland. This may be because PBR exists in greater quantities in salivary gland. The results of the present study showed that the level of PBR mRNA in salivary gland was higher than that in Cx. This result was supported by the receptor binding assay, which showed that the Kd value of [3H] PK11195 was similar in Cx and salivary gland, but that that of Bmax was higher in salivary gland than that in Cx [23].

The inhibitory effect of METH-withdrawal stress on salivary secretion was greater than that of steroids or GABA$_A$ agonist. It is possible that METH-withdrawal stress inhibits salivary secretion not only via steroid biosynthesis, but also some other pathway. It is known that PBR regulates Ca^{2+} efflux from mitochondria and apoptosis [1]. Further study is required to elucidate PBR-regulated Ca^{2+} efflux from mitochondria in rat salivary gland. In addition, METH-withdrawal stress may influence other organs. An earlier study showed that steroidogenic enzyme CYP11A1, but not 3β-HSD or CYP17a, was found in salivary gland by RT-PCR and Western blot [13]. These results suggest that PRG is the only convincing candidate for steroid production in salivary gland. However, Fig.4 shows that some steroid hormones which are specifically produced in each organ such as adrenal gland, testis, ovary or ovarian follicle, inhibit salivary secretion, not only PRG. These results indicate that these steroids move hematogenously to the salivary gland, where they affect secretion. Further study is needed to determine the levels of these steroids in blood plasma.

Fig. 5 shows a schema of the pathways activated by METH-withdrawal stress in salivary gland based on

a)

DBI

β-Actin

saline Meth   saline Meth   saline Meth   saline Meth
Cx   PG   SMG   SLG

b)
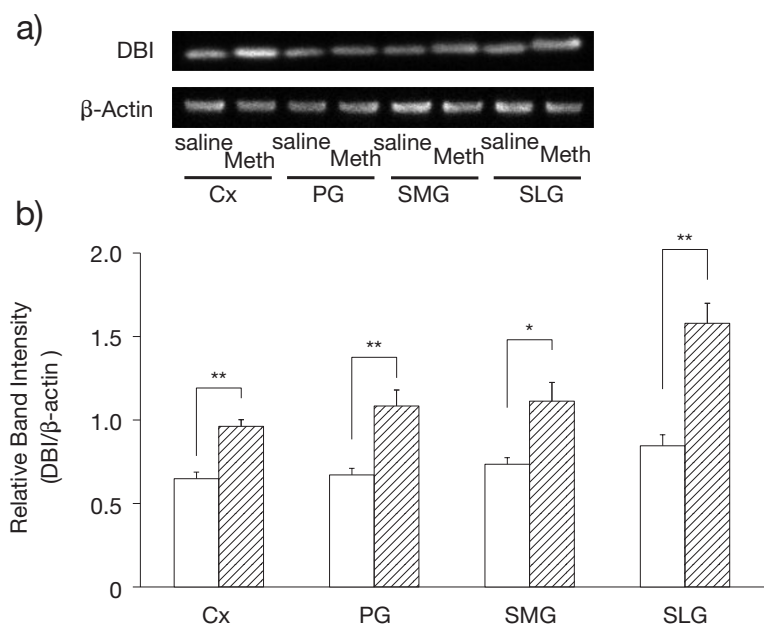
Fig. 3 Increased expression of DBI protein in the salivary gland by METH-withdrawal stress using Western blot analysis. a) Bands indicate DBI (10 kD), and β-actin (42 kD) in AG, Cx, PG, SMG, and SLG in METH withdrawal–stress rats and control rats. Western blot data are representative of 5 experiments. b) Graph shows DBI protein levels in Cx, PG, SMG, and SLG in METH withdrawal–stress rats (stripe column) and control rats (open column). Semiquantitative analysis was performed at 5 different densities of Western blot using a CS Analyzer. Data were normalized to the density of the β-actin band. Results represent mean with S.E.M. of data from 5 rats. *,** Significantly different from values in control group treated with saline at $P < 0.05$, $P < 0.01$.

Fig. 4 Effects of steroids on CCh-induced salivary secretion in rat submandibular gland perfusion system. 10^{-6}M allopregnanolone (AP), testosterone (TES), hydrocortisone (HC), progesterone (PRO), estradiol (EST), or PRG was administered over 10-min period commencing 5 min prior to administration of 10^{-6} M CCh over 5 min in rat SMG. Saliva was collected at 30 min after stimulation. Net weight of collected saliva was determined and expressed as percentage of secretion induced by CCh alone. Secretion induced by CCh alone was 19.7 ± 1.9 ml/30 min (n = 5). Data represent mean ± S.E.M. of 4 experiments. ** Significantly different from CCh alone at $P < 0.01$.
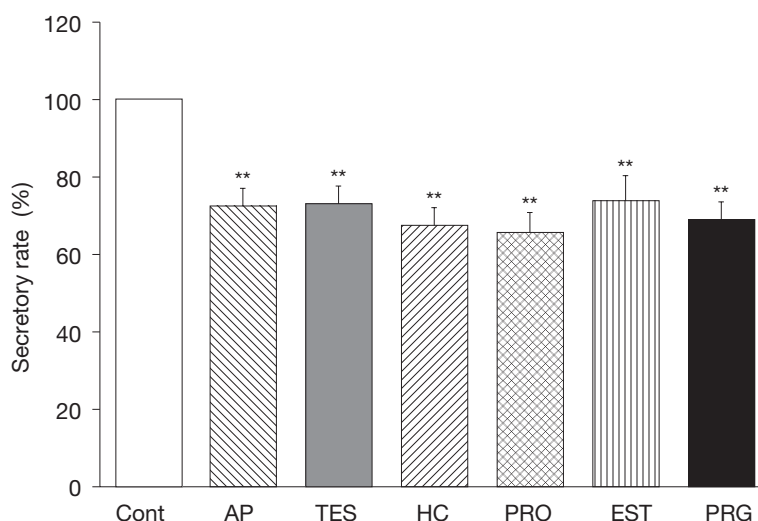
the results of present and previous our studies. DBI mRNA expression was upregulated with an increase in PACAP through activation of PAC1-R. DBI mainly acts as a PBR agonist, stimulating neurosteroid synthesis in mitochondria. PRG, a neurosteroid and first metabolite formed from cholesterol by CYP11A1, acts as an allosteric agonist of GABA$_A$-R. Therefore, overexpression of PRG enhances GABA$_A$-R function, resulting in an increase in intracellular Cl$^-$ levels, followed by termination of Ca^{2+} release from intracellular calcium stores and suppression of Cl$^-$ efflux throughout Ca^{2+}-dependent Cl$^-$ channels in the apical membrane of acinar cells. These events lead to a decline in transcellular movement of water, causing a decrease in salivary secretion.

In conclusion, our present results suggest that METH-withdrawal stress activates steroid biosynthesis via a PACAP-DBI pathway in salivary gland, resulting in inhibition of salivary secretion.

REFERENCES

1) Azarashvili T, Grachev D, Krestinina O, Evtodienko Y, Yurkov I, Papadopoulos V, *et al*. The peripheral-type benzodiazepine receptor is involved in control of Ca^{2+}-induced permeability transition pore opening in rat brain mitochondria. Cell Calcium 2007; 42: 27–39.

2) Di Cugno F, Perec CJ, Tocci AA. Salivary secretion and dental caries experience in drug addicts. Arch Oral Biol 1981; 26: 363–367.

3) Katsura M, Hara A, Higo A, Tarumi C, Hibino Y, Ohkuma S. Continuous treatment with morphine increases diazepam binding inhibitor mRNA in mouse brain. J Neurochem 1998; 71: 2638–2641.

4) Katsura M, Ohkuma S, Tsujimura A, Kuriyama K. Increase of diazepam binding inhibitor mRNA levels in the brains of chronically ethanol-treated and -withdrawn mice. J Pharmacol Exp Ther 1995; 273: 1529–1533.
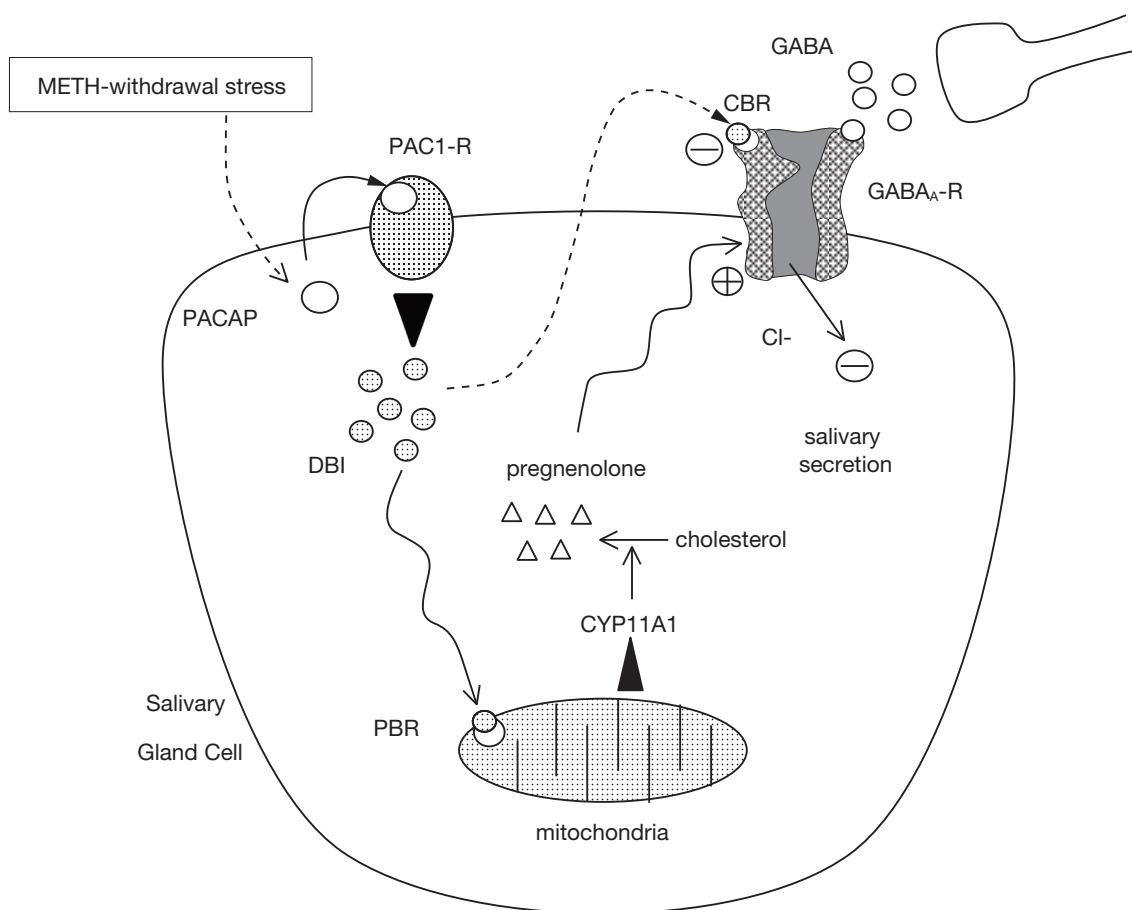
Fig. 5　Inhibitory mechanism of METH-withdrawal stress in rat salivary gland cell
METH: methamphetamine, CBR: central-type benzodiazepine receptor, PBR: peripheral type benzodiazepine receptor, DBI: diazepam-binding inhibitor, PACAP: pituitary adenylate cyclase–activating polypeptide, PAC1-R: PACAP type I receptor, GABAA-R: gamma-aminobutyric acid A receptor

5) Katsura M, Ohkuma S, Xu J, Hibino Y, Tsujimura A, Kuriyama K. Continuous treatment with nicotine increases diazepam binding inhibitor (DBI) and its mRNA in the mouse brain. Brain Res Mol Brain Res 1998; 55: 345–349.

6) Kawaguchi M, Ouchi K, Ohse S, Baba Y. In vivo and in vitro studies on receptive mechanisms for benzodiazepines in rat parotid gland. Dent Jpn 1995; 32: 38–40.

7) Kawaguchi, M., Yamagishi, H Coupling of benzodiazepine and GABA(A) receptors in the salivary glands is a factor of drug-induced xerostomia. Int. Acad. Biomed. Drug Res 1996; 11: 291–296.

8) Kujirai M, Sawaki K, Kawaguchi M. Inhibitory effect of diazepam on muscarinic receptor-stimulated inositol 1,4,5-trisphosphate production in rat parotid acinar cells. Br J Pharmacol 2002; 137: 945–952.

9) Liu X, Li Y, Zhou L, Chen H, Su Z, Hao W. Conditioned place preference associates with the mRNA expression of diazepam binding inhibitor in brain regions of the addicted rat during withdrawal. Brain Res Mol Brain Res 2005; 137: 47–54.

10) Maeda T, Kiguchi N, Fukazawa Y, Yamamoto A, Ozaki M, Kishioka S. Peroxisome proliferator-activated receptor gamma activation relieves expression of behavioral sensitization to methamphetamine in mice. Neuropsychopharmacology 2007; 32: 1133–1140.

11) Majewska MD. Neurosteroids: endogenous bimodal modulators of the GABAA receptor Mechanism of action and physiological significance. Prog Neurobiol 1992; 38: 379–395.

12) Masmoudi-Kouki O, Gandolfo P, Leprince J, Vaudry D, Pelletier G, Fournier A, *et al.* PACAP stimulates biosynthesis and release of endozepines from rat astrocytes. Ann N Y Acad Sci 2006; 1070: 411–416.

13) Miyashita T, Okubo M, Shinomiya T, Nakagawa K, Kawaguchi M. Pregnenolone biosynthesis in the rat salivary gland and its inhibitory effect on secretion. J Pharmacol Sci 2011; 115: 56–62.

14) Neess D, Kiilerich P, Sandberg MB, Helledie T, Nielsen R, Mandrup S. ACBP-a PPAR and SREBP modulated housekeeping gene. Mol Cell Biochem 2006; 284, 149-57.

15) Okubo M, Kawaguchi M. Inhibitory regulation of amylase release in rat parotid acinar cells by benzodiazepine receptors. Eur J Pharmacol 1998; 359: 243–249.

16) Okubo M, Kawaguchi M. Co-operative effect between GABA_A receptors and central-type benzodiazepine receptors on amylase release in rat parotid acinar cells. J Pharmacol Sci. 2010; 112: 247–250.

17) Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere JJ, Lindemann P, *et al.* Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci 2006; 27: 402–409.

18) Rey C, Mauduit C, Naureils O, Benahmed M, Louisot P, Gasnier F. Up-regulation of mitochondrial peripheral benzodiazepine receptor expression by tumor necrosis factor alpha in testicular leydig cells. Possible involvement in cell survival. Biochem Pharmacol 2000; 60: 1639–1646.

19) Saini T, Edwards PC, Kimmes NS, Carroll LR, Shaner JW, Dowd FJ. Etiology of xerostomia and dental caries among methamphetamine abusers. Oral Health Prev Dent 2005; 3: 189–195.

20) Tamaki R, Yoshikawa M, Shinomiya T, Andoh H, Kawaguchi M, Hashimoto A, Chronic administration of methamphetamine increases the mRNA expression of diazepam binding inhibitor in rat brain. Tokai J Exp Clin Med 2008; 33: 46–50.

21) Tsukagoshi E, Kawaguchi M, Shinomiya T, Yoshikawa M, Kawano

T, Okubo M Diazepam Enhances Production of Diazepam-Binding Inhibitor (DBI), a Negative Saliva Secretion Regulator, Localized in Rat Salivary Gland. J Pharmacol Sci 2011; 115: 221−229.

22) Watanabe M, Yamagishi-Wang H, Kawaguchi M Lowered susceptibility of muscarinic receptor involved in salivary secretion of streptozotocin-induced diabetic rats. Jpn J Pharmacol 2001; 87: 117−124.

23) Yamagishi H, Kawaguchi M Characterization of central- and peripheral- type benzodiazepine receptors in rat salivary glands. Biochem Pharmacol 1998; 55: 209−214.