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RESTORATION OF MORPHINE-INDUCED ALTERATIONS IN RAT SUBMANDIBULAR GLAND FUNCTION BY N-METHYL-D-ASPARTATE AGONIST

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The effects of morphine, 1-aminocyclobutane-cis-1,3-dicarboxylic (ACBD; NMDA agonist) and 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphoric acid (CPP; NMDA antagonist) and their concurrent therapy on rat submandibular secretory function were studied. Pure submandibular saliva was collected intraorally by micro polyethylene cannula from anaesthetized rats using pilocarpine as secretagogue. Intraperitoneal injection of morphine (6 mg/kg) induced significant inhibition of salivary flow rate, total protein, calcium, and TGF-β1 concentrations. Administration of ACBD (10 mg/kg) and CPP (10 mg/kg) alone did not influence secretion of submandibular glands. In combination therapy, coadministration of CPP with morphine did not influence morphine-induced changes in salivary function while ABCD could restore all morphine-induced changes. In combination treatment, ACBD prevented morphine-induced reduction of flow rate, total protein, calcium, and TGF-β1 and reached control levels. It is concluded that morphine-induced alterations in submandibular gland function are mediated through NMDA receptors.

Keywords: 1-aminocyclobutane-cis-1,3-dicarboxylic – 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphoric acid – N-methyl-D-aspartate – morphine – submandibular gland – saliva

INTRODUCTION

Salivary glands have become a useful model for the study of some basic problems in physiology, such as transepithelial salt and water transport, protein synthesis and exocytosis, the neuropharmacology of autonomic nerves and receptors, and stimulus secretion coupling [1]. The saliva consists of water, electrolytes, proteins and enzymes that are under the control of autonomic nerves. There are two major intracellular mechanisms for secretion, including generation of cAMP and breakdown of

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plasma membrane polyphosphoinositides play the main role [13, 22]. Opioids have been found to affect these intracellular mediator systems and calcium operating in salivary glands [27, 33, 54]. The significant distribution of morphine to submandibular glands of rats 10–160 min after its intraperitoneal (i.p.) injection was confirmed by immunohistochemistry [60]. An association between morphine administration and dry mouth was reported in human during a study involving 199 cancer patients. The mechanism of morphine-induced dryness of the mouth is unclear [59]. Recent study indicated that morphine administration to rats affects their submandibular gland function and changes composition of saliva [2].

The NMDA-type glutamate receptor has been implicated in the behavioral processes of learning and memory [49], opioid analgesia [14], locomotion [52], and there are reports that elevated Ca^{2+} is associated with clinically defined affective disorder [30]. The acute interaction between opioid and NMDA receptors on nociception provides new rationale for beneficial adjunction of NMDA receptor antagonist. It has been indicated that the antinociceptive effects of morphine and fentanyl are blunted by concomitant NMDA-dependent opposing effects which are only revealed when the predominant antinociceptive effect is sharply blocked by naloxone [18]. The role of amino acids in salivation and the localization of their receptors in the rat salivary gland were investigated. It has been suggested that glutamate dose not act alone but with other substances such as peptide, and/or other amino acids [51]. It has been suggested that glutamatergic inputs regulate the activity of superior salivary nucleus (SSN) neurons and play a role in the regulation of pontin preganglionic (PPG) function [35]. The glutamatergic input to pontin preganglionic neurons of the superior salivary nucleus in rat was examined. It has been shown that NMDA receptor activation and glutamatergic stimulation influence superior salivary nucleus neurons [37].

Among the salivary glands, the submandibular produces of more than 70% of saliva excreted regulatory in mouth and it consists of water, protein, enzymes, and electrolytes [22]. Growth factors are mainly excreted from this gland [3] and among them, transforming growth factor β1 (TGF-β1) has been characterized as an injury-related factor, based on the observation that it is strongly regulated in many acute or chronic central nervous system disorder [57]. Proper secretion of submandibular saliva has a beneficiary role in oral and total body health [1, 55]. With respect to these findings, we were interested to examine whether morphine could interact with the NMDA system in rat submandibular gland function.

MATERIALS AND METHODS

Materials

1-aminocyclobutane-cis-1,3-dicarboxylic (ACBD; NMDA agonist) and 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphoric acid (CPP; NMDA antagonist) from Tocris (USA), pilocarpine, pentobarbital sodium, standard bovine serum albumin,

and folin phenol reagent from Sigma-Aldrich (UK), and TGF-β1 ELISA determination kit from Bio Source System (USA) were used in this study.

Animals

Adult male, Sprague-Dawley rats, weighing 150–250 g were used in this study. Following several days of acclimatization, rats were randomly distributed into control and experimental groups. Rats had free access to stock laboratory diet and water. They were caged in an environment at 21–24 °C with a light/dark cycle of 14/10 h starting at 6:00 A.M. Animals were randomly distributed into experimental groups with five animals in each group.

First experiment

Rats were divided into two groups. Animals in the treated groups were administered a single intraperitoneal (ip) injection of morphine (6 mg/kg) and control received the equivolume amount of saline [2].

Second experiment

Rats were divided into three groups. Animals in the first and second groups were administered ip ACBD (10 mg/km) and CPP (10 mg/kg) respectively. Controls received the equivolume amount of saline [4].

Third experiment

Animals were divided into four groups and received morphine (6 mg/kg) alone, ACBD (10 mg/kg) plus morphine (6 mg/kg), CPP (10 mg/kg) plus morphine (6 mg/kg) respectively. The fourth group was assigned as control and received only saline.

Saliva collection

All of the saliva collections were done at 9:00 A.M. to reduce the diurnal effect on saliva secretions. All animals were anaesthetized by ip injection of sodium pentobarbital (55 mg/kg) repeated (30 mg/kg) after 15 minutes to maintain complete general anesthesia until end of the experiment [5]. They were then secured in a supine position with taps, and tracheotomized to facilitate respiration during experiment. Both submandibular ducts were cannulated intraorally with polyethylene tubes [61].

All dissections were performed with the aid of a dissecting microscope. As secretory stimuli, pilocarpine was dissolved in isotonic saline and administered ip at the dose of 8 mg/kg. With the exception of the initial two drops which were discarded, saliva was collected for 30 minutes into pre-weighed stoppered microtubes kept in ice and stored at –20 °C for subsequent analyses.

Gland removal and determination of flow rate

After saliva collection, the right and left submandibular glands were dissected free of connective tissues and were weighted. Flow rate was calculated from the sample weight, the wet weight of the gland with the assumption that the specific gravity of saliva is 1.0 [56]. All salivary parameters were normalized for salivary flow rate and then reported.

Protein determination

The concentration of protein in the saliva was determined by the Lowry procedure using bovine serum albumin as the standard [40].

Calcium, sodium and potassium measurement

These were determined in saliva by flame atomic absorption spectrophotometer (Shimadzu 680-AA, Japan) as described previously [25].

TGF-β1 determination

Concentrations of TGF-β1 in saliva were measured using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

The statistical analysis was performed by SPSS version 11.5. Continuous data are expressed as mean ± SE. Data were compared using one way ANOVA and the posthoc Tukey multicomparison tests. A P value <0.05 was considered to be statistically significant.

RESULTS

Flow rate

Administration of morphine decreased ($P < 0.01$) salivary flow rate in comparison to controls (29.66 ± 1.78 vs. 39.9 ± 2.39 mg/g/min). Administration of ACBD (41.62 ± 2.50 µl/mg/min) and CPP (37.02 ± 2.22 mg/g/min) alone did not significantly change salivary flow rate in comparison to control levels (39.9 ± 2.39 mg/g/min). Coadministration of ACBD with morphine (37.9 ± 2.27 mg/g/min) recovered ($P < 0.01$) morphine-induced (29.66 ± 1.78 mg/g/min) decrease in flow rate and reached to control levels (39.9 ± 2.39 mg/g/min). Coadministration of CPP with morphine (25.82 ± 1.55 mg/g/min) did not significantly affect morphine-induced (29.66 ± 1.78 mg/g/min) change in flow rate (Fig. 1).

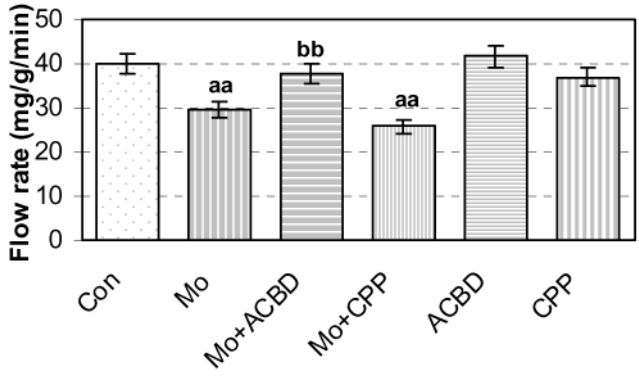

Fig. 1. Effects of morphine (Mo), ACBD, and CPP alone or in combination on salivary flow rate. [aa] means that the difference between control (Con) and treated groups is significant at $P < 0.01$. [bb] means that the difference between morphine and morphine plus ACBD or CPP treated groups is significant at $P < 0.01$

Protein concentration

Administration of morphine increased ($P < 0.01$) salivary protein in comparison to control (12.5 ± 0.75 vs. 6.82 ± 0.41 mg/ml/flow rate). Administration of ACBD (7.1 ± 0.43 mg/ml/flow rate) and CPP (7.35 ± 0.44 mg/ml/flow rate) alone did not significantly change salivary control (6.82 ± 0.41 mg/ml/flow rate) protein concentration. Coadministration of ACBD with morphine (9.82 ± 0.59 mg/ml/flow rate) partly recovered ($P < 0.01$) morphine-induced (12.5 ± 0.75 mg/ml/flow rate) increase in protein but did not reach to control levels (6.82 ± 0.41 mg/ml/flow rate). Coadministration of CPP with morphine (11.61 ± 0.70 mg/ml/flow rate) did not significantly affect morphine-induced (12.5 ± 0.75 mg/ml/flow rate) change in protein concentration (Fig. 2).

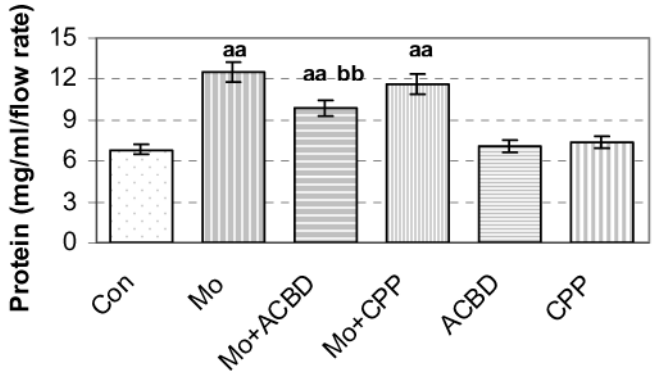

Fig. 2. Effects of morphine (Mo), ACBD, and CPP alone or in combination on salivary protein concentration. [aa] means that the difference between control (Con) and treated groups is significant at $P < 0.01$. [bb] means that the difference between morphine and morphine plus ACBD or CPP treated groups is significant at $P < 0.01$

Calcium concentration

Administration of morphine increased ($P < 0.1$) salivary calcium in comparison to control (10.86 ± 0.65 vs. 4.9 ± 0.29 mEq/L/flow rate). Administration of ACBD (5.2 ± 0.31 mEq/L/flow rate) and CPP (5.5 ± 0.33 mEq/L/flow rate) alone did not significantly change salivary control (4.9 ± 0.29 mEq/L/flow rate) calcium concentration. Coadministration of ACBD with morphine (7.38 ± 0.44 mEq/L/flow rate) partly recovered ($P < 0.01$) morphine-induced (10.86 ± 0.65 mEq/L/flow rate) increase in calcium but did not reach to control levels (4.9 ± 0.29 mEq/L/flow rate). Coadministration of CPP with morphine (9.85 ± 0.59 mEq/L/flow rate) did not significantly affect morphine-induced change (10.86 ± 0.65 mEq/L/flow rate) in calcium concentration (Fig. 3).

TGF-β1 concentration

Administration of morphine increased ($P < 0.1$) salivary TGF-β1 in comparison to control (0.76 ± 0.05 vs. 0.57 ± 0.03 pg/L/flow rate). Administration of ACBD (0.56 ± 0.03 pg/L/flow rate) and CPP (0.55 ± 0.03 pg/L/flow rate) alone did not significantly change salivary control TGF-β1 concentration (0.57 ± 0.03 pg/L/flow rate). Coadministration of ACBD with morphine (0.57 ± 0.03 pg/L/flow rate) completely recovered ($P < 0.01$) morphine-induced (0.76 ± 0.05 pg/L/flow rate) increase in TGF-β1 but did not reach to control levels (0.57 ± 0.03 pg/L/flow rate). Coadministration of CPP with morphine (0.79 ± 0.05 pg/L/flow rate) did not significantly affect morphine-induced (0.76 ± 0.05 pg/L/flow rate) change in TGF-β1 concentration (Fig. 4).

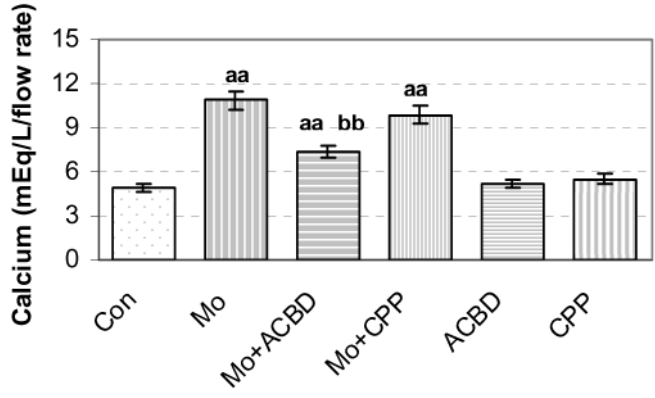

Fig. 3. Effects of morphine (Mo), ACBD, and CPP alone or in combination on salivary calcium concentration. [aa] means that the difference between control (Con) and treated groups is significant at $P < 0.01$. [bb] means that the difference between morphine and morphine plus ACBD or CPP treated groups is significant at $P < 0.01$

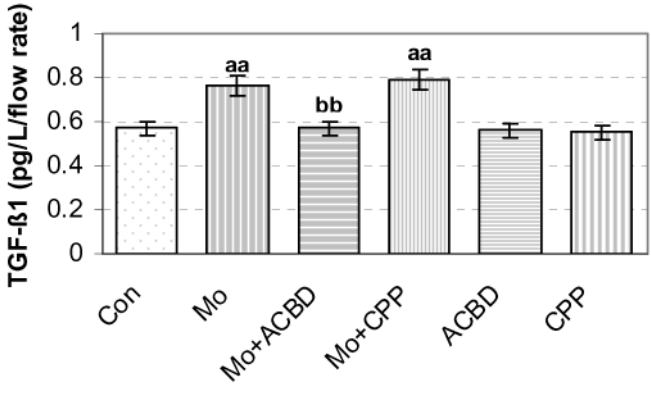

Fig. 4. Effects of morphine (Mo), ACBD, and CPP alone or in combination on salivary TGF-β1 concentration. [aa] means that the difference between control (Con) and treated groups is significant at $P < 0.01$. [bb] means that the difference between morphine and morphine plus ACBD or CPP treated groups is significant at $P < 0.01$

Sodium and potassium concentration

Administration of morphine alone or in combination did not significantly change salivary control concentrations of sodium and potassium. The control values for sodium and potassium were 4.94 ± 0.30 and 4.90 ± 0.29, respectively.

DISCUSSION

The overall results indicated that administration of NMDA receptor agonist and antagonist themselves do not significantly alter salivary function. In the meantime, administration of morphine stimulated secretion of calcium, total protein and TGF-β1 but inhibited flow rate. Furthermore coadministration of NMDA agonist with morphine restored morphine-induced alterations in salivary function.

There are two major transduction mechanisms operative in salivary gland acinar cells; one generates cAMP and the other breaks down plasma membrane polyphosphoinositides. Secretion of proteins including growth factors like TGF-β1 into saliva is mediated to a greater extent through β-adrenergic receptors and cAMP-dependent pathway, while salivary flow rate is mainly controlled by cellular phosphoinositide cascade and mainly inositol triphosphate [13, 17]. It is well known that calcium is the common intracellular mediator and increased intracellular calcium concentration acts as a signal or second messenger in many functions of salivary glands [10]. As shown, concentration of calcium was markedly affected in submandibular gland by morphine administration. Regulation of calcium channels by all opioid receptors has been determined in submandibular ganglion neurons using the patch-clamp technique [23]. Thus, interaction of morphine with calcium homeostasis can be the first mechanism that can be explained for the observed effects by morphine in salivary function. Supporting this finding, it has been reported that morphine reduces salivary flow rate examined in rat [2] and human [59]. As another explanation for effects of morphine, it should be noted that the endogenous opioid peptide systems contribute in the mediation, modulation and regulation of stress responses by the autonomic nervous system [21]. Therefore, morphine by affecting pre- or post-synaptic opioid receptors might influence sympathetic and parasympathetic functions in this gland. In addition, coupling of spinal mu- and delta-opioid receptors to phospholipase C has been shown to increase phosphoinositide metabolism and the generation of inositol phosphate that can influence salivary function [48]. There are also evidences that morphine interacts with cAMP-dependent process of salivary protein secretion. They have been reported that activation of opioid receptors is accompanied with cAMP accumulation [11, 38] and accentuating the ability of the cAMP-dependent pathways [16, 32] especially through adenylyl cyclase supersensitization [12, 58]. This up-regulation involves increased concentration of adenylyl cyclase, cAMP-dependent protein kinase A, and other components of this signaling pathway [44]. Another mechanism for increasing in cAMP accumulation could be due to the ability of opioid receptor to stimulate adenylyl cyclase via Gs proteins [26].

In the present study TGF-β1 is also increased by morphine administration. The previous studies have shown the amplifying effect of morphine on the release of TGF-β1 that is thought to be mediated through opioid system. Given the fact that TGF-β1 has a potent immunosuppressive effect, morphine-potentiated release of TGF-β1 from peripheral blood mononuclear cells may be involved in the immunomodulatory activity ascribed to morphine [19]. Morphine-induced

macrophage apoptosis is also mediated through downstream signaling involving TGF-β1 and nitric oxide (NO) production [15].

In explanation of results obtained further coadministration of ACBD and CPP weith morphine, there is evidence that NMDA agonist does not itself affect salivary flow despite application of electrical stimulation, while at very high concentrations inhibits salivary secretion [51]. In the submandibular gland, non-cholinergic vasodilation of vascular beds, elicited by parasympathetic nerve stimulation, seems to be due to NO action (24, 50) and NO might act as a neurotransmitter in the regulation of blood flow and secretion [39]. It is clearly shown that the inhibitory effect of morphine is potentiated in the presence of NOS inhibitor and diminished by NO precursor [2]. Recent in vitro [62] and in vivo [45] findings indicate the coupling of NMDA receptor activation with the synthesis of NO. It acts as a retrograde messenger to enhance presynaptic glutamate release [6]. The coupling of NMDA receptor activation with NO synthesis occurs in brain regions that contain NO synthesizing neurons [28]. In addition, there is evidence that administration of NMDA into hippocampal [36] and striatal [29] tissue increases NO production, and it is generally considered that behavioral effects of NMDA and related compounds may involve a final common pathway that is influenced by the intracellular flow of Ca^{2+} and its subsequent role in production of NO [43]. On the other hand, mu opioid receptors modulate NMDA receptor mediated responses in nucleus accumbens neurons. The nucleus accumbens may play a major role in opiate dependence, and central NMDA receptors are reported to influence opiate tolerance and dependence [41]. Supporting this theory, there are several evidences indicating protective effects of NO in salivary glands [7, 8, 9, 46, 47]. In addition, opioid treatment leads to PKC activation and translocation [20], which phosphorylates the NMDA receptor-gated Ca channel, resulting in removal of the Mg blockade and potentiation of NMDA-receptor activity [34]. Opening of these channels leads to Ca influx and increased intracellular Ca concentrations, producing several effects such as positive feedback regulation of PKC activity [42]. It further promotes influx of Ca through voltage-gated Ca channels [53] which may then accentuate PKC activation and activation of nitric oxide synthase (NOS). Ca influx may also directly result in activation of AC and increase in cAMP levels [31].

The collective results of this study confirm that morphine is able to alter rat submandibular gland function while these alterations can be restored using NMDA agonist.

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REFERENCES

1. Abdollahi, M., Radfar, M. (2003) A review of drug-induced oral reactions. *J. Contemp. Dent. Pract. 4,* 10–31.
2. Abdollahi, M., Safarhamidi, H. (2002) Protection by nitric oxide of morphine-induced inhibition of rat submandibular gland function. *Pharmacol. Res. 45,* 87–92.
3. Abdollahi, M., Simaiee, B. (2003) Stimulation by theophylline and sildenafil of rat submandibular secretion of protein, epidermal growth factor and flow rate. *Pharmacol. Res. 48,* 445–449.
4. Abdollahi, M., Sarrafzadeh, J., Nikfar, S., Roshanzamir, F. (2003) Mechanism of aspartame-induced antinociception in mice. *Indian J. Pharmacol. 35,* 37–41.
5. Abdollahi, M., Dehpour, A. R., Baharnouri, G. (1998) Effects of rubidium on the secretory function of the rat submandibular gland. *Toxic. Subs. Mech. 17,* 121–131.
6. Abdollahi, M., Dehpour, A. R. (2000) Kazemian P. Alteration by cadmium of rat submandibular gland secretory function and the role of the L-arginine/nitric oxide pathway. *Pharmacol. Res. 42.*
7. Abdollahi, M., Dehpour, A. R., Fooladgar, M. (1997) Alteration of rat submandibulary gland secretion of protein, calcium and N-acetyl-beta-D-glucosaminidase activity by lead. *Gen. Pharmacol. 29,* 675–680.
8. Abdollahi, M., Sharifzadeh, M., Marzban, H., Abri, G. H., Torab-Jahromi, M. (1999) Alteration by lead acetate of rats submandibular gland morphology and ultrastructure. *Toxic. Subs. Mech. 18,* 139–148.
9. Abdollahi, M., Dehpour, A. R., Shafayee, F. (2000) L-arginine/nitric oxide pathway and interaction with lead acetate on rat submandibulary gland function. *Pharmacol. Toxicol. 87,* 198–203.
10. Aub, D. L., Putney, J. W. (1987) Mobilization of intracellular calcium by methacholine and inositol 1,4,5-triphosphate in rat parotid acinar cells. *J. Dent. Res. 66,* 547–551.
11. Avidor-Reiss, T., Nevo, I., Levy, R., Pfeuffer, T., Vogel, Z. (1996) Chronic opioid treatment induces adenylyl cyclase V superactivation. Involvement of Gbetagamma. *J. Biol. Chem. 271,* 21309–21315.
12. Avidor-Reiss, T., Bayewitch, M., Levy, R., Matus-Leibovitch, N., Nevo, I., Vogel, Z. (1995) Adenylylcyclase supersensitization in mu-opioid receptor-transfected Chinese hamster ovary cells following chronic opioid treatment. *J. Biol. Chem. 270,* 29732–29738.
13. Baum, B. J. (1987) Neurotransmitter control of secretion. *J. Dent. Res. 66,* 628–632.
14. Ben-Eliyahu, S., Marek, P., Vaccarino, A. L., Mogil, J. S., Sternberg, W. F., Liebeskind, J. C. (1992) The NMDA receptor antagonist MK-801 prevents long-lasting non-associative morphine tolerance in the rat. *Brain Res. 575,* 304–308.
15. Bhat, R. S., Bhaskaran, M., Mongia, A., Hitosugi, N., Singhal, P. C. (2004) Morphine-induced macrophage apoptosis: oxidative stress and strategies for modulation. *J. Leukoc. Biol. 75,* 1131–1138.
16. Blake, A. D., Bot, G., Freeman, J. C., Reisine, T. (1997) Differential opioid agonist regulation of the mouse mu opioid receptor. *J. Biol. Chem. 272,* 782–790.
17. Castle, J. D., Arvan, P., Cameron, R. (1987) Protein production and secretion in exocrine cell. *J. Dent. Res. 66,* 633–637.
18. Celerier, E., Laulin, J. P., Larcher, A., Moal, M. L., Simonnet, G. (1999) Evidence for opiate-activated NMDA processes masking opiate analgesia rats. *Brain Res.* 18–25.
19. Chao, C. C., Hu, S., Molitor, T. W., Zhou, Y., Murtaugh, M. P., Tsang, M., Peterson, P. K. (1992) Morphine potentiates transforming growth factor-beta release from human peripheral blood mononuclear cell cultures. *J. Pharmacol. Exp. Ther. 262,* 19–24.
20. Chen, L., Huang, L. Y. (1991) Sustained potentiation of NMDA receptor-mediated glutamate responses through activation of protein kinase C by a mu opioid. *Neuron. 7,* 319–326.
21. Drolet, G., Dumont, E. C., Gosselin, I., Kinkead, R., Laforest, S., Trottier, J. F. (2001) Role of endogenous opioid system in the regulation of the stress response. *Prog. Neuropsychopharmacol. Biol. Psychiatry 25,* 729–741.
22. Edgar, W. M. (1992) Saliva: its secretion, composition and functions. *Br. Dent. J. 172,* 305–312.

23. Endoh, T., Suzuki, T. (1998) The regulation manner of opioid receptors on distinct types of calcium channels in hamster submandibular ganglion cells. *Arch. Oral. Biol. 43,* 221–233.
24. Edwards, A. V., Garrett, J. R. (1993) Nitric oxide-related vasodilator responses to parasympathetic stimulation of the submandibular gland in the cat. *J. Physiol. 464,* 379–392.
25. Fernandez, F. J., Khan, H. L. (1971) Clinical methods for atomic absorption spectroscopy. *Clin. Chem. Newslett. 24,* 190–195.
26. Fields, A., Sarne, Y. (1997) The stimulatory effect of opioids on cyclic AMP production in SK-N-SH cells is mediated by calcium ions. *Life Sci. 61,* 595–602.
27. Fundytus, M. E., Coderre, T. J. (1996) Chronic inhibition of intracellular Ca^{2+} release or protein kinase C activation significantly reduces the development of morphine dependence. *Eur. J. Pharmacol. 300,* 173–181.
28. Garthwaite, J. (1991) Glutamate, nitric oxide and cell-cell signaling in the nervous system. *Trends Neurosci. 14,* 60–67.
29. Hanania, T., Johnson, K. M. (1998) Regulation of neurotransmitter release by endogenous nitric oxide in striatal slices. *Eur. J. Pharmacol. 359,* 111–117.
30. Helmeste, D. M., Tang, S. W. (1998) The role of calcium in the etiology of the affective disorders. *Jpn. J. Pharmacol. 77,* 107–116.
31. Hirst, R. A., Lambert, D. G. (1995) Adenylyl cyclase in SH-SY5Y human neuroblastoma cells is regulated by intra-and extracellular calcium. *Biochem. Pharmacol. 49,* 1633–1640.
32. Jin, W., Terman, G. W., Chavkin, C. (1997) Kappa opioid receptor tolerance in the guinea pig hippocampus. *J. Pharmacol. Exp. Ther. 281,* 123–128.
33. Johnson, P. S., Wang, J. B., Wang, W. F., Uhl, G. R. (1994) Expressed mu opiate receptor couples to adenylate cyclase and phosphatidyl inositol turnover. *Neuroreport 5,* 507–509.
34. Kitamura, Y., Miyazaki, A., Yamanaka, Y., Nomura, Y. (1993) Stimulatory effects of protein kinase C and calmodulin kinase II on N-methyl-D-aspartate receptor/channels in the postsynaptic density of rat brain. *J. Neurochem. 61,* 100–109.
35. Kobayashi, M., Nemoto, T., Nagata, H., Konno, A., Chiba, T. (1997) Immunohistochemical studies on glutamatergic, GABAergic and glycinergic axon varicosities presynaptic to parasympathetic preganglionic neurons in the superioi salivatory nucleus of the rat. *Brain Res. 766,* 72–82.
36. Kojima, H., Nakatsubo, N., Kikuchi, K., Urano, Y., Higuchi, T., Tanaka, J., Kudo, Y., Nagano, T. (1998) Direct evidence of NO production in rat hippocampus and cortex using a new fluorescent indicator: DAF-2 DA. *Neuroreport 9,* 3345–3348.
37. Lin, L. H., Agassandian, K., Fujiyama, F., Kaneko, T., Talmas, W. T. (2003) Evidence for a glutamatergic input to pontin preganglionic neurons of the superior salivary nucleus in rat. *J. Chem. Neuroanat. 25,* 261–268.
38. Liu, J. G., Anand, K. J. (2001) Protein kinases modulate the cellular adaptations associated with opioidtolerance and dependence. *Brain Res. Brain Res. Rev. 38,* 1–19.
39. Lohinai, Z., Szekeli, A. D., Soos, L., Feher, E. (1995) Distribution of nitric oxide synthase containing elements in the feline submandibular gland. *Neurosci. Lett. 192,* 9–12.
40. Lowry, O. H., Roserbrough, N. J., Farr, A. L., Randell, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem. 193,* 265–275.
41. Martin, G., Nie, Z., Siggins, G. R. (1997) mu-Opioid receptors modulate NMDA receptor-mediated responses in nucleus accumbens neurons. *J. Neurosci. 17,* 11–22.
42. Mayer, D. J., Mao, J., Price, D. D. (1995) The development of morphine tolerance and dependence is associated with translocation of protein kinase. *C. Pain. 61,* 365–374.
43. Menendez, L., Hidalgo, A., Baamonde, A. (1997) Spinal calmodulin inhibitors reduce N-methyl-D-aspartate- and septide-induced nociceptive behavior. *Eur. J. Pharmacol. 335,* 9–14.
44. Nestler, E. J., Aghajanian, G. K. (1997) Molecular and cellular basis of addiction. *Science 278,* 58–63.
45. Podhorna, J., Brown, R. E. (2000) Interaction between N-methyl-D-aspartate and nitric oxide in the modulation of ultrasonic vocalizations of infant rats. *Eur. J. Pharmacol. 408,* 265–271.

46. Rezaie, S., Rezaie, A., Minaiee, B., Khorasani, R., Abdollahi, M. (2005) On the relation of nitric oxide to nifedipine-induced gingival hyperplasia and impaired submandibular glands function in rats *in vivo*. *Fund. Clin. Pharmacol. 19,* 65–71.
47. Ahmed, A. O., Sharifzadeh, M., Nikfar, S., Jamshidi, H. R., Abdollahi, M. (2006) Prevention by L-arginine/nitric oxide of chlordiazepoxide-induced toxic reactions in the rat salivary gland. *Toxicol. Mech. Methods 16,* 331–337.
48. Sanchez-Blazquez, P., Rodriguez-Diaz, M., Frejo, M. T., Garzon, J. (1999) Stimulation of mu- and delta-opioid receptors enhances phosphoinositide metabolism in mouse spinal cord: evidence for subtypes of delta-receptors. *Eur. J. Neurosci. 11,* 2059–2064.
49. Sanger, D. J., Joly, D. (1991) The effects of NMDA antagonists on punished exploration in mice. *Behav. Pharmacol. 2,* 57–63.
50. Schachter, M., Matthews, B., Bhoola, K. D. (1992) Evidence that nitric oxide or a related substance is a neurovasodilator in the submandibular gland of cat. *Agents. Actions 38,* 366–370.
51. Shida, T., Kondo, E., Ueda, Y., Takai, N., Yoshida, Y., Araki, T., Kiyama, H., Tohyama, M. (1995) Role of amino acids in salivation and the localization of their receptors in the rat salivary gland. *Brain. Res. Mol. Brain. Res. 33,* 261–268.
52. Shoaib, M., Schindler, C. W., Goldberg, S. R., Pauly, J. R. (1997) Behavioural and biochemical adaptations to nicotine in rats: influence of MK801, an NMDA receptor antagonist. *Psychopharmacology* (Berl.) *134,* 121–130.
53. Smart, D., Lambert, D. G. (1996) delta-Opioids stimulate inositol 1,4,5-trisphosphate formation, and so mobilize Ca^{2+} from intracellular stores, in undifferentiated NG108-15 cells. *J. Neurochem. 66,* 1462–1467.
54. Smith, F. L., Lohmann, A. B., Dewey, W. L. (1999) Involvement of phospholipid signal transduction pathways in morphine tolerance in mice. *Br. J. Pharmacol. 128,* 220–226.
55. Sreebny, L. M. (2000) Saliva in health and disease: an appraisal and update. *Int. Dent. J. 50,* 140–161.
56. Suddick, R. P., Shannon, I. L. (1970) Salivary Na^+, K^+, and Cl-secretion rates relationship to a fluid generation mechanism. *Jpn. J. Physiol. 20,* 540–549.
57. Vivien, D., Ali, C. (2005) Transforming growth factor-beta signaling in brain disorder. *Cytokine Growth Factor Rev.* 2005 [Epud ahead of print]
58. Wang, Z., Bilsky, E. J., Porreca, F., Sadee, W. (1994) Constitutive μ opioid receptor activation as a regulatory mechanism underlying narcotic tlerance and dependence. *Life Sci.* 54.
59. White, I. D., Hoskin, P. J., Hanks, G. W., Bliss, J. M. (1989) Morphine and dryness of the mouth. *B. M. J. 298,* 1222–1223.
60. Xu, Z., Wu, J., Wang, B., Wang, W. (1998) Distribution of morphine in acute morphine-treated rats. *Hua Xi Yi Ke Da Xue Xue Bao 29,* 29–32.
61. Yoshida, Y., Sprecher, R. L., Schneyer, C. A., Schneyer, L. H. (1967) Role of α-receptors in sympathetic regulation of electrolytes in rat submaxillary saliva. *Proc. Soc. Exp. Biol. Med. 126,* 912–916.
62. Zhang, J., Synder, S. H. (1995) Nitric oxide in the nervous system. *Annu. Rev. Pharmacol. Toxicol. 35,* 213–233.