ELSEVIER

European Journal of Pharmacology 276 (1995) 3–7

ejp

Effects of adenosine receptor agonists and antagonists on physostigmine-induced yawning

Mohammad-Reza Zarrindast [a,*], Reza Adeli [a], Sedigheh Hosseini-semnani [b], Mohammad Sharifzadeh [a]

[a] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*
[b] *Department of Physiology, College of Medical Science, University of Tarbiat Modarres, Tehran, Iran*

Received 14 December 1994; accepted 16 December 1994

Abstract

The effect of adenosine receptor agonists and antagonists on physostigmine-induced yawning was investigated in intact or cannulated rats. Intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) administration of physostigmine to rats induced yawning dose dependently. I.p. or i.c.v. treatment of the animals with atropine, theophylline, 5-*N*-ethylcarboxamidoadenosine (NECA) or N^6-cyclohexyladenosine reduced the yawning induced by i.p. injection of physostigmine. I.p. administration of theophylline decreased the yawning induced by i.c.v injection of physostigmine. The inhibitory action of N^6-cyclohexyladenosine (i.p.) also was decreased by 8-phenyltheophylline (i.p.) pretreatment. It is concluded that yawning induced by a central cholinergic mechanism and a central adenosine mechanism interacts with the cholinergic-induced behaviour.

Keywords: Yawning; Physostigmine; Atropine; Adenosine receptor agonist; Adenosine receptor antagonist; (Rat)

1. Introduction

Yawning behaviour has been suggested to be a physiological response associated with fatique and recovery from stress (Barbizet, 1958; Stoessl et al., 1987). Although yawning is a curious and still little understood behaviour which is displayed in many vertebrate species (Baenninger, 1987), it is nonetheless a discrete and easily quantifiable behaviour that can be used as a model for the understanding of various central nervous system functions. Current models based on pharmacological experiments suggest that cholinergic and dopaminergic systems induce yawning behaviour in rats. Interaction between cholinergic and dopaminergic systems in yawning behaviour (Mogilnicka et al., 1984; Zarrindast and Poursoltan, 1989) and also between dopaminergic (Brown et al., 1991) or cholinergic systems (Brown et al., 1990) with an adenosine mechanism has been shown.

Adenosine plays a functionally important role in nervous tissue as a regulator of neural activity (Phillis and Wu, 1981; Snyder, 1985). Adenosine inhibits neural firing and release of neurotransmitters such as acetylcholine, γ-aminobutyric acid, dopamine, norepinephrine and glutamate in the brain (Harms et al., 1979; Fredholm and Hedqvist, 1980; Stone, 1981; Dolphin and Archer, 1983; Spignoli et al., 1984; O'Regan and Phillis, 1987). Adenosine receptors have been divided into A_1 and A_2 subtypes by Van Calker et al. (1979), based on the ability of adenosine analogs to inhibit or stimulate adenyl cyclase. Both receptors are present in the central nervous system (Daly, 1985; Fredholm, 1982). A_1 adenosine receptors widely distribute in brain, whereas A_2 receptor sites are localised in dopamine-rich brain areas such as striatum, nucleus accumbens and olfactory tubercle (Bruns et al., 1986). The striatum may be one of the sites involved in yawning induced by drugs (Yamada et al., 1986). A_1 and A_2 adenosine receptors are present in the striatum, which has been shown to regulate acetylcholine release (Brown et al., 1990). Our previous work has shown that the adenosine receptor antagonist, theophylline, inhibits yawning behaviour in rats (Zarrindast and Poursoltan, 1989; Zarrindast and Nasir, 1991).

In the present study, we have tested the effects of

* Corresponding author.

Elsevier Science B.V.
SSDI 0014-2999(94)00767-5

adenosine receptor agonists and antagonists on yawning induced by the anticholinesterase agent, physostigmine.

2. Materials and methods

2.1. Animals

Male albino rats weighing 250–300 g were used for all experiments. The animals were housed in wire cages in groups of five, under controlled conditions of temperature (20 ± 2°C) and light (12 h/12 h light/dark cycle). The rats were allowed free access to food and water, except during the time of experiments.

2.2. Chronic guide cannula implantation

For intracerebroventricular (i.c.v.) injection, the rats were anaesthetized with ketamine hydrochloride (65 mg/kg i.p.) plus Rompun (14 mg/kg i.p.), were placed in a stereotaxic frame (David Koft Instruments, USA) and a stainless steel guide cannula was implanted in the left lateral ventricle at the following coordinates: AP = −0.8 mm, L = −1.6 mm (both with respect to bregma) and V = 3.5 from the dura. For the recovery period, the animals were caged individually for a period of 7 days. The drugs were injected in a volume of 3 μl over a period of 2 min, by means of an internal cannula (28 gauge) connected by polyethylene tubing to a 25-μl Hamilton syringe and the injection cannula was left in place for a further 1 min before being slowly withdrawn.

2.3. Behavioural observations

The animals were placed individually in clear glass beakers to allow them to adjust for 15 min before injection of drugs. Immediately after drug administration, the number of yawns was counted by direct observation and recorded for a period of 60 min.

Statistical analysis of the data was performed with an analysis of variance (ANOVA) followed by the Newman-Keuls test. Differences with $P < 0.05$ were considered statistically significant.

2.4. Drugs

The drugs used were atropine sulphate (E. Merck, Germany), Rompun (Bayer Leverkusen, Germany), ketamine hydrochloride (Parke-Davis, France), physostigmine, theophylline, 8-phenyltheophylline, N^6-cyclohexyladenosine and 5-*N*-ethylcarboxamidoadenosine (Sigma, UK). 8-Phenyltheophylline was dissolved in a drop of ethylenediamine then diluted with water. The other drugs were dissolved in distilled water. For i.p. injection, the drugs were given in a total volume of 10 ml/kg.

3. Results

3.1. Physostigmine-induced yawning in rats

Intraperitoneal (i.p.) injection of different doses of the anticholinesterase, physostigmine (0.05, 0.1, 0.2 and 0.3 mg/kg) induced dose-dependent yawning episodes in rats. Maximum response was achieved with 0.2 mg/kg of the drug (Fig. 1).

Intracerebroventricular (i.c.v.) administration of different doses of physostigmine (0.5, 1, 3 and 5 μg/rat) also induced yawning in the animals in a dose-related manner. The maximum effect was obtained with 3 μg/rat (Fig. 2).

3.2. Effects of atropine and adenosine agents on physostigmine-induced yawning

I.p. pretreatment of animals with the antimuscarinic drug, atropine (1, 2 and 4 mg/kg, 30 min), the adenosine receptor antagonist, theophylline (12.5, 25, 50 and 100 mg/kg, 60 min), or the adenosine receptor agonist, NECA (0.0005, 0.001, 0.005, 0.01 and 0.05 mg/kg, 60 min), decreased the response induced by i.p. administration of physostigmine (0.2 mg/kg). The inhibition of physostigmine-induced yawning by the drugs was dose related (Table 1).

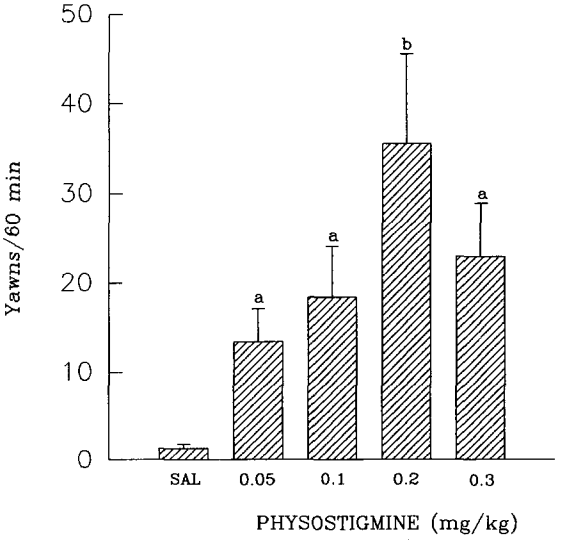

Fig. 1. Yawning induced by administration of physostigmine in rats. Animals were injected intraperitoneally (i.p.) with saline (SAL; 5 ml/kg) or with different doses of physostigmine. The number of yawns was recorded for 60 min. Each point is the mean ± S.E.M. for 7 rats. $^aP < 0.05$, $^bP < 0.01$ significantly different from saline-treated group.

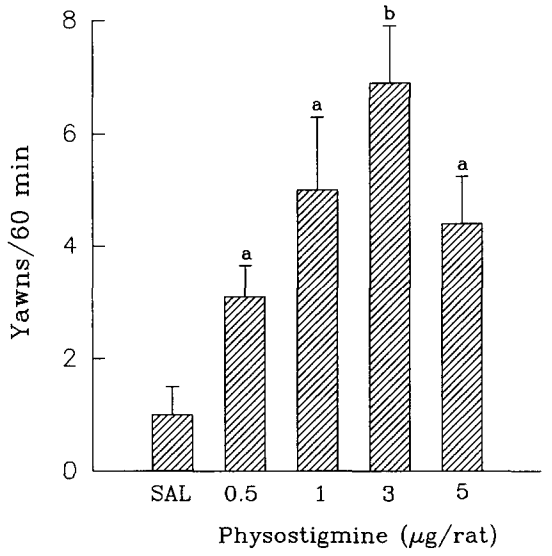

Fig. 2. Yawning induced by intracerebroventricular (i.c.v.) injection of physostigmine in rats. The animals were given either saline (SAL; 3 μl/rat) or different doses of physostigmine. Each point is the mean ± S.E.M. of 7 experiments. $^{a}P < 0.05$, $^{b}P < 0.01$ significantly different from saline control animals.

3.3. Effect of N^6-cyclohexyladenosine in the presence or absence of 8-phenyltheophylline on physostigmine-induced yawning

When animals were pretreated (i.p.) with the adenosine receptor agonist N^6-cyclohexyladenosine (0.05, 0.1, 0.25, 0.5 and 1 mg/kg) 60 min prior to physostigmine (0.2 mg/kg), the yawning induced by the cholinergic drug was reduced dose dependently. The inhibition of the yawning induced by N^6-cyclohexyladenosine (0.25, 0.5 and 1 mg/kg) was decreased by 8-phenyltheophylline (2 mg/kg, 60 min) pretreatment. The adenosine receptor antagonist, 8-phenyltheophylline (0.5–6 mg/kg i.p.), alone did not alter the yawning induced by physostigmine (Table 2).

Table 1
Effects of intraperitoneal (i.p.) injection of theophylline, NECA and atropine on yawning induced by physostigmine (0.2 mg/kg i.p.)

Treatment	mg/kg	Yawns/60 min (mean ± S.E.M.)
Saline	5 ml	32.5 ± 5.7
Theophylline	12.5	21.8 ± 5.4 [a]
Theophylline	25	15.8 ± 2.2 [b]
Theophylline	50	8.1 ± 1.8 [b]
Theophylline	100	0.4 ± 0.3 [b]
NECA	0.0005	28.8 ± 5.5
NECA	0.001	19.7 ± 3.2 [a]
NECA	0.005	20.3 ± 3.4 [a]
NECA	0.01	7.9 ± 2.0 [b]
NECA	0.05	2.0 ± 1.1 [b]
Atropine	1	18.2 ± 3.6 [b]
Atropine	2	7.2 ± 2.9 [b]
Atropine	4	2.3 ± 1.3 [b]

Animals were pretreated (i.p.) either with saline, theophylline or NECA 60 min and atropine 30 min prior to physostigmine (0.2 mg/kg i.p.) injection. Yawning was recorded 60 min after the physostigmine administration for a period of 60 min. Each point is the mean ± S.E.M. of at least 9 experiments.
[a] $P < 0.05$, [b] $P < 0.01$ significantly different from control (saline + physostigmine) group.

Table 2
Effect of i.p. administration of N^6-cyclohexyladenosine (CHA) in the presence or absence of 8-phenyltheophylline (8-PT) on yawning induced by physostigmine (0.2 mg/kg i.p.)

Treatment	mg/kg	Yawns/60 min (mean ± S.E.M.)
Saline	5 ml	27.8 ± 6.1
8-PT	0.5	16.8 ± 5.0
8-PT	1	15.6 ± 2.8
8-PT	2	20.2 ± 3.1
8-PT	4	27.6 ± 6.2
8-PT	6	24.4 ± 7.5
Saline	5 ml	32.5 ± 5.7
CHA	0.05	16.1 ± 4.8 [b]
CHA	0.1	13.3 ± 3.4 [b]
CHA	0.25	9.9 ± 3.7 [b]
CHA	0.5	3.2 ± 1.2 [b]
CHA	1	0.1 ± 0.1 [b]
CHA	0.25	
+ 8-PT	2	18.5 ± 5.4
CHA	0.5	
+ 8-PT	2	15.5 ± 2.5 [a]
CHA	1	
+ 8-PT	2	15.0 ± 4.6 [b]

Animals were treated either with saline or 8-PT, CHA and CHA + 8-PT 60 min before physostigmine injection. Other details as in Table 1.
[a] $P < 0.05$, [b] $P < 0.01$ significantly different from control group.

3.4. Effects of i.p. injection of theophylline on yawning induced by i.c.v. administration of physostigmine

Intraperitoneal treatment of animals with theophylline (10, 20 and 30 mg/kg) 60 min before i.c.v. administration of physostigmine (3 μg/rat) decreased the yawning induced by the cholinesterase inhibitor. The inhibitory response to theophylline was dose dependent (Fig. 3).

3.5. Effects of i.c.v. administration of atropine or adenosine drugs on yawning induced by i.p. injection of physostigmine

In animals treated intracerebroventricularly with atropine (0.001, 0.003 and 0.005 ng/rat), theophylline (0.005, 0.007 and 0.01 ng/rat), N^6-cyclohexyladenosine (1, 5 and 10 ng/rat) or NECA (0.05, 0.5 and 5 ng/rat) immediately after i.p. injection of physostigmine (0.2

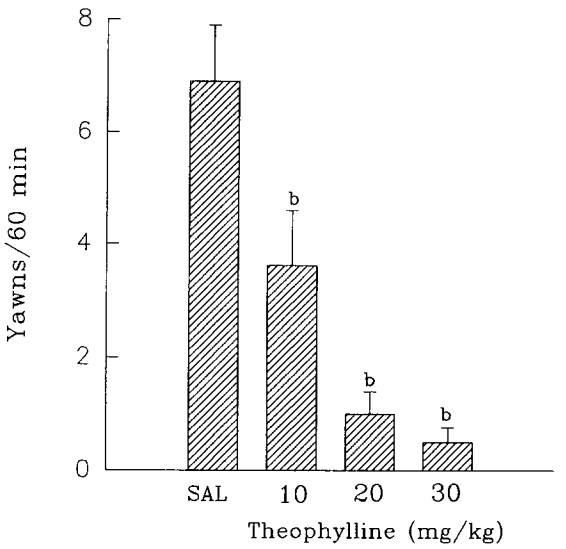

Fig. 3. Effect of theophylline on physostigmine-induced yawning. The animals were pretreated (i.p.) either with saline (SAL; 5 ml/kg) or with different doses of theophylline, 60 min before physostigmine (3 μg/rat, i.c.v.) administration. Each point is the mean ± S.E.M. for 7 animals. $^{b}P < 0.01$ significantly different from control group.

mg/kg), the yawning induced by the cholinergic drug was reduced (Table 3).

4. Discussion

In the present work, both intracerebroventricular (i.c.v.) or intraperitoneal (i.p.) administration of the cholinesterase inhibitor, physostigmine, induced dose-dependent yawning. The response induced by i.p. injection of the cholinergic drug was decreased by both i.p. or i.c.v. administration of the muscarinic receptor antagonist, atropine. The data indicate that a central cholinergic stimulation mechanism is involved in physostigmine-induced yawning. This is in agreement with a previous report that activation of cholinergic mechanisms can induce yawning (Zarrindast and Poursoltan, 1989). Septal and striatal dopamine D_2 receptors has been suggested to be involved in yawning in rats (Yamada et al., 1986). It has been also shown that yawning induced by a dopaminergic mechanism is mediated through cholinergic activation (Carlsson, 1975; Di Chiara et al., 1976). Accordingly, it can be suggested that septal and striatal cholinergic systems are the sites of physostigmine-induced yawning.

Table 3
Effects of intracerebroventricular (i.c.v.) injection of atropine, adenosine receptor agonists and antagonist on yawning induced by physostigmine (0.2 mg/kg i.p.)

Treatment	ng/rat	Yawns/60 min (mean ± S.E.M.)
Saline	3 μl	20.1 ± 3.5
Theophylline	0.005	13.1 ± 2.8 [a]
Theophylline	0.007	7.0 ± 1.2 [b]
Theophylline	0.01	4.7 ± 1.1 [b]
CHA	1	13.1 ± 1.6 [a]
CHA	5	10.8 ± 1.3 [a]
CHA	10	9.2 ± 1.2 [b]
NECA	0.05	7.7 ± 0.8 [b]
NECA	0.5	10.7 ± 1.3 [a]
NECA	5	11.7 ± 2.5 [a]
Atropine	0.001	20.2 ± 3.9
Atropine	0.003	13.4 ± 2.4
Atropine	0.005	3.5 ± 0.7 [b]

Animals were injected intracerebrally (i.c.v.) with saline, theophylline, N^6-cyclohexyladenosine (CHA), NECA and atropine immediately after physostigmine (0.2 mg/kg i.p.) administration. Yawning episodes were recorded immediately after injection of the drugs for a period of 60 min. Each point is the mean ± S.E.M. for at least 7 animals.
[a] $P < 0.05$, [b] $P < 0.01$ significantly different from control (saline + physostigmine) group.

The present results show that the adenosine receptor agonists, N^6-cyclohexyladenosine (Moos et al., 1985) and NECA (Heffner et al., 1989), when administered either peripherally or centrally, decreased the behaviour induced by a cholinergic agent, physostigmine, suggesting interactions of central adenosine mechanism(s) with cholinergic-induced yawning behavior.

Adenosine actions have been detected at both presynaptic and postsynaptic sites (Proctor and Dunwiddie, 1983; Lee et al., 1984; Schubert and Lee, 1986). Both adenosine A_1 and A_2 receptors are present in the striatum, and are localized to cholinergic nerve terminals. The former have been shown to inhibit acetylcholine release. In contrast, adenosine A_2 receptor cause stimulation of acetylcholine release (Richardson and Brown, 1987; Brown et al., 1990). It has been shown that N^6-cyclohexyladenosine and NECA have affinity for both adenosine A_1 and A_2 receptors (Stone, 1985). Considering the cholinergic nature of yawning induced by physostigmine (Urba-Holmgren et al., 1977; Zarrindast and Poursoltan, 1989), a possibility may exist that A_1 activation by N^6-cyclohexyladenosine or NECA decreases the release of acetylcholine and in turn reduces the behaviour. The adenosine A_1 receptor antagonist, 8-phenyltheophylline, did not alter the physostigmine response, but prevented the inhibition of the yawning induced by N^6-cyclohexyladenosine. This may support the suggestion that adenosine A_1 activation causes a decrease in the cholinergic-induced yawning. In contrast to 8-phenyltheophylline, the adenosine receptor antagonist, theophylline, decreased the yawning induced by physostigmine. Theophylline has been proposed to be an adenosine receptor antagonist (Bruns et al., 1986) which may exert a greater A_2 antagonist effect (Ferre et al., 1991). Thus there is the possibility that blockade of adenosine A_2 receptors by theophylline decreased the release of acetylcholine. This possibility is supported by the finding of Brown et al. (1990) that A_2 stimulation is able to increase acetyl-

choline release in the striatum. Since N^6-cyclohexyladenosine and NECA have no selective affinity for adenosine A_1 or A_2 receptors (Stone, 1985), selective adenosine receptor agonists may be needed to clarify the exact mechanism involved. High doses of theophylline inhibit phosphodiesterase; considering that activation of adenosine A_1 receptors decreases cAMP levels (Van Calker et al., 1979), should stimulation of adenosine A_1 receptors be responsible for inhibition of yawning, the increase in cAMP caused by theophylline cannot be part of the mechanism of the effect on yawning.

References

Baenninger, R., 1987, Some comparative aspects of yawning in beta splendens, homo sapiens, panthera leo and papio sphinx, J. Comp. Physiol. 4, 349.

Barbizet, J., 1958, Yawning, J. Neurol. Neurosurg. Psychiatry 21, 203.

Brown, S.J., S. James, M. Redington and P.J. Richardson, 1990, Both A_1 and A_{2a} purine receptors regulate striatal acetylcholine release, J. Neuropharmacol. 55, 31.

Brown, S.J., R. Gill, J.L. Evenden, S.D. Iversen and P.J. Richardson, 1991, Striatal A_2 receptor regulates apomorphine-induced turning in rats with unilateral dopamine denervation, Psychopharmacology 103, 78.

Bruns, R.F., G.H. Lu and T.A. Pugsley, 1986, Characterization of the A_1 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes, Mol. Pharmacol. 29, 331.

Carlsson, A., 1975, Receptor-mediated control of dopamine metabolism, in: Pre- and Postsynaptic Receptors, eds. E. Usdin and W.E. Bunney (Marcel Dekker, New York) p. 49.

Daly, J.V., 1985, Adenosine receptors, Adv. Cyclic Nucleotide Protein Phosphorylation Res. 19, 29.

Di Chiara, G., M.L. Porceddu, L., Vargiu, A. Argiolas and G.L. Gessa, 1976, Evidence for dopamine receptors mediating sedation in the mouse brain, Nature 264, 564.

Dolphin, A.C. and E.R. Archer, 1983, An adenosine agonist inhibits and a cyclic AMP analogue enhances the release of glutamate but not GABA from slices of rat dentate gyrus, Neurosci. Lett. 43, 49.

Ferre, S., M. Herrera-Marshitz, M. Grabowska-Anden, M. Casas, U. Ungerstedt and N.E. Anden, 1991, Postsynaptic dopamine receptor/adenosine interaction. II. Postsynaptic dopamine agonism and adenosine antagonism of methylxanthines in short-term reserpinized mice, Eur. J. Pharmacol. 192, 31.

Fredholm, B.B., 1982, Adenosine actions and adenosine receptors after 1 week treatment with caffeine, Acta Physiol. Scand. 115, 283.

Fredholm, B.B. and P. Hedqvist, 1980, Modulation of neurotransmission by purine nucleotides and nucleotides, Biochem. Pharmacol. 29, 1635.

Harms, H.H., G. Warden and A.H. Mulder, 1979, Effects of adenosine on depolarization-induced release of various radiolabelled neurotransmitters from slices of rat corpus striatum, Neuropharmacology 18, 577.

Heffner, T.G., J.N. Wiley, A.E. Williams, R.F. Bruns, L.L. Coughenour and D.A. Downs, 1989, Comparison of the behavioural effects of adenosine agonists and dopamine antagonists in mice, Psychopharmacology 98, 31.

Lee, K.S., P. Shubert and V. Heinemann, 1984, The anticonvulsive action of adenosine: a postsynaptic dendritic action by a possible endogenous anticonvulsant, Brain Res. 321, 160.

Mogilnicka, E., C.G. Boissard and A. Delini-Stula, 1984, Effects of apomorphine, TL-99 and 3-ppp on yawning in rats, Neuropharmacology 23, 19.

Moos, W.H., D.S. Szotek and R.F. Bruns, 1985, N^6-Cycloalkyladenosine: potent, A_1 selective adenosine agonists, J. Med. Chem. 28, 1383.

O'Regan, M.H. and J.W. Phillis, 1987, Pertusis toxin blocks the inhibitory effects of adenosine on rat cerebral cortical neurons, Brain Res. 436, 380.

Phillis, J.W. and P.H. Wu, 1981, The role of adenosine and its nucleotides in central synaptic transmission, Prog. Neurobiol. 16, 187.

Proctor, W.R. and T.V. Dunwiddie, 1983, Adenosine inhibits calcium spikes in hippocampal pyramidal neurons in vitro, Neurosci. Lett. 35, 197.

Richardson, P.J. and S.J. Brown, 1987, ATP release from affinity purified rat cholinergic nerve terminals, J. Neurochem. 48, 622.

Schubert, P. and K.S. Lee, 1986, Nonsynaptic modulation of repetitive firing by adenosine is antagonized by 4- aminopyridine in a rat hippocampal slice, Neurosci. Lett. 67, 334.

Snyder, S.H., 1985, Adenosine as a neuromodulator, Annu. Rev. Neurosci. 8, 103.

Spignoli, G., F. Pedata and G. Pepeu, 1984, A_1 and A_2 adenosine receptors modulate acetylcholine release from brain slices, Eur. J. Pharmacol. 97, 341.

Stoessl, A.J., C.T. Dourish and S.D. Iversen, 1987, Apomorphine-induced yawning in rats is abolished by bilateral 6- hydroxydopamine lesions of substantia nigra, Psychopharmacology 93, 336.

Stone, T.W., 1981, Physiological roles for adenosine and ATP in the nervous system, Neuroscience 6, 523.

Stone, T.W., 1985, Purines, Pharmacology and Physiological Roles, ed. T.W. Stone (VCH, Weinheim) p. 1.

Urba-Holmgren, R., R.M. Gonzalez and B. Holmgren, 1977, Is yawning a cholinergic response?, Nature 267, 261.

Van Calker, P., M. Muller and B. Hamprecht, 1979, Adenosine regulates via two different types of receptors the accumulation of cAMP in cultured brain cells, J. Neurochem. 33, 999.

Yamada, K., M. Tanaka, K. Shibata and T. Furukawa, 1986, Involvement of septal and striatal dopamine D-2 receptors in yawning behaviour in rats, Psychopharmacology 90, 9.

Zarrindast, M.R. and T. Nasir, 1991, Methylxanthines-induced attenuation of pecking in chickens, Br. J. Pharmacol. 104, 327.

Zarrindast, M.R. and M. Poursoltan, 1989, Interaction of drugs acting on central dopamine receptors and cholinoceptors on yawning responses in the rat induced by apomorphine, bromocriptine or physostigmine, Br. J. Pharmacol. 96, 843.