ELSEVIER

European Journal of Pharmacology 425 (2001) 51–55

ejp

www.elsevier.com/locate/ejphar

Role of adenosine and P2 receptors in the penile tumescence in anesthetized dogs

Tsunehisa Noto *, Hirotaka Inoue, Hideki Mochida, Kohei Kikkawa

Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-2-50, Kawagishi, Toda, Saitama 335-8505, Japan

Received 8 March 2001; received in revised form 25 June 2001; accepted 26 June 2001

Abstract

We studied the role of adenosine and P2 receptors in the pelvic nerve stimulation-induced penile tumescence in anesthetized dogs. A local intracavernous injection of adenosine induced the tumescence, which was abolished by intracavernous 8-(*p*-sulfophenyl)theophylline (8-SPT), an unspecific adenosine receptor antagonist, and by 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)phenol (ZM241385), an adenosine A_{2A} receptor antagonist. ATP also induced the tumescence, which was diminished by 8-SPT, but not by reactive blue-2, a P2 receptor antagonist. Neither intracavernous β, γ-meATP nor $ADP_{\beta}S$, P2X and P2Y receptor agonists, induced tumescence. N^{G}-nitro-L-arginine (L-NAME), a nitric oxide synthase inhibitor, and T-1032, a phosphodiesterase type V inhibitor, had no effects on the tumescence induced by adenosine. 8-SPT and reactive blue-2 had no effects on the tumescence induced by pelvic nerve stimulation. These results show that although exogenous adenosine and ATP induce tumescence, neither the adenosine nor the P2 receptor is involved in the tumescence induced by pelvic nerve stimulation in anesthetized dogs. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: ATP; Pelvic nerve; T-1032

1. Introduction

Many studies have shown that nitric oxide (NO) synthesized from L-arginine mainly mediates the relaxation of penile corpus cavernosum muscle and the intracavernous pressor response to nerve stimulation in a variety of mammals (Anderson, 1993; Ignarro et al., 1990; Trigo-Rocha et al., 1993). Although it has also been reported that several other endogenous neurotransmitters such as adenosine, ATP, vasoactive intestinal polypeptide and calcitonin gene-related peptide exist in the corpus cavernosum, their roles in erection are still unclear (Andersson and Wagner, 1995). Adenosine and ATP are reported to induce tumescence when they are injected into the corpus cavernosa in dogs and to relax the isolated corpus smooth muscles from humans, rats and rabbits (Chiang et al., 1994; Filippi et al., 1999; Gur and Ozturk, 2000; Ragazzi et al., 1996; Takahashi et al., 1992a,b; Wu et al., 1993). In addition, the nerve stimulation-induced relaxation of the isolated corpus smooth muscle is inhibited by 3,7-dimethyl-1-propargylxanthine (DMPX), an adenosine A_2 receptor antagonist (Chiang et al., 1994). These data suggest that adenosine and ATP may contribute to physiological penile erection, but there are no reports about their roles in erection in in vivo models.

We now studied the roles of adenosine and ATP in pelvic nerve-stimulated tumescence in anesthetized dogs using some receptor antagonists.

2. Materials and methods

This project was approved by the Ethical Committee at Tanabe Seiyaku and all efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1. Penile tumescence in anesthetized dogs

Experiments were performed on male mongrel dogs weighing between 12 and 21 kg. The dogs were anesthetized with pentobarbital sodium (30 mg/kg i.v. bolus injection, followed by 4.5 mg/kg/h i.v. infusion). An endotracheal tube was placed for ventilation (15 ml/kg/stroke, 20 strokes/min) with room air. The femoral artery was cannulated for continuous blood pressure monitoring.

* Corresponding author. Tel.: +81-48-433-8042; fax: +81-48-433-8157.

E-mail address: t-noto@tanabe.co.jp (T. Noto).

0014-2999/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(01)01167-0

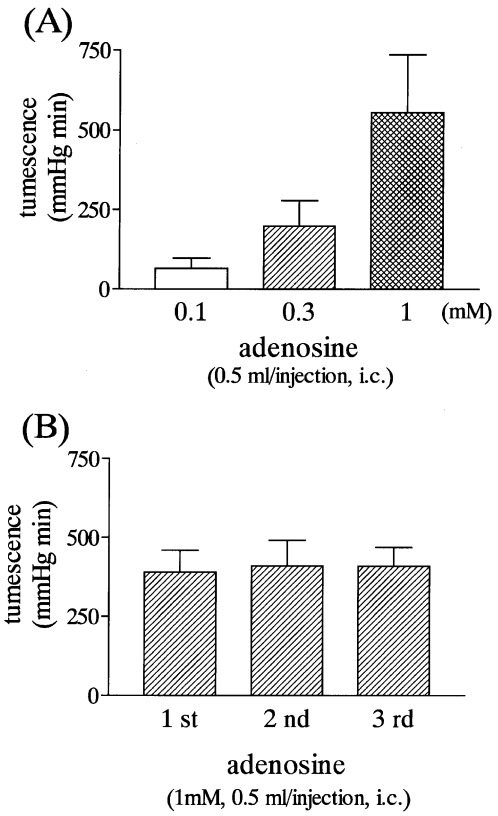

Fig. 1. (A) Intracavernous adenosine injection (0.5 ml/injection) induced tumescence dose-dependently in anesthetized dogs ($n = 6$). (B) Repeated adenosine (1 mM, i.c., three times) induced tumescence to the same extent ($n = 3$).

The left pelvic nerves, located superior and lateral to the prostate, were carefully isolated and placed on a bipolar electrode (IMT-1530; Inter Medical, Nagoya, Japan) connected to an electronic stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). Two 23-gauge venous needles were placed in the corpus cavernosum on the left side (~ 1 cm apart): one was connected to the pressure transducer (TP-400T, Nihon Kohden) and the recorder (WR3701; Graphtec, Tokyo, Japan) for recording intracavernous pressure, and the other was used for intracavernous injection of drugs. Adenosine receptor antagonists were administered intracavernously 5 min before the tumescence. The effective doses of these drugs were determined from a preliminary dose–response study. N^G-nitro-L-arginine (L-NAME) was also injected intracavernously at a concentration of 30 mM as previously reported (Trigo-Rocha et al., 1993). Reactive blue-2 was administered intracavernously at a concentration of 10 mM, which was supposed to be enough to block the P2 receptor, because the dose of 20 μM (1/500 of 10 mM) was previously reported to be effective in an in vitro study with corpus cavernosum (Shalev et al., 1999). T-1032 was given intravenously 5 min before the tumescence. The pelvic nerves were stimulated by electrical square pulses (200-μs pulse width) of 10 V at frequencies from 3.3 to 5 Hz for a period of 40 s. For quantitative determination of the tumescence, we measured the area under the curve and expressed it as millimeters of mercury multiplied by minutes. The tumescences were repeated three times in each animal at intervals of 30 min.

2.2. Statistical analysis

The results were expressed as means ± S.E. Statistical analyses were done with Student's *t*-test for paired data,

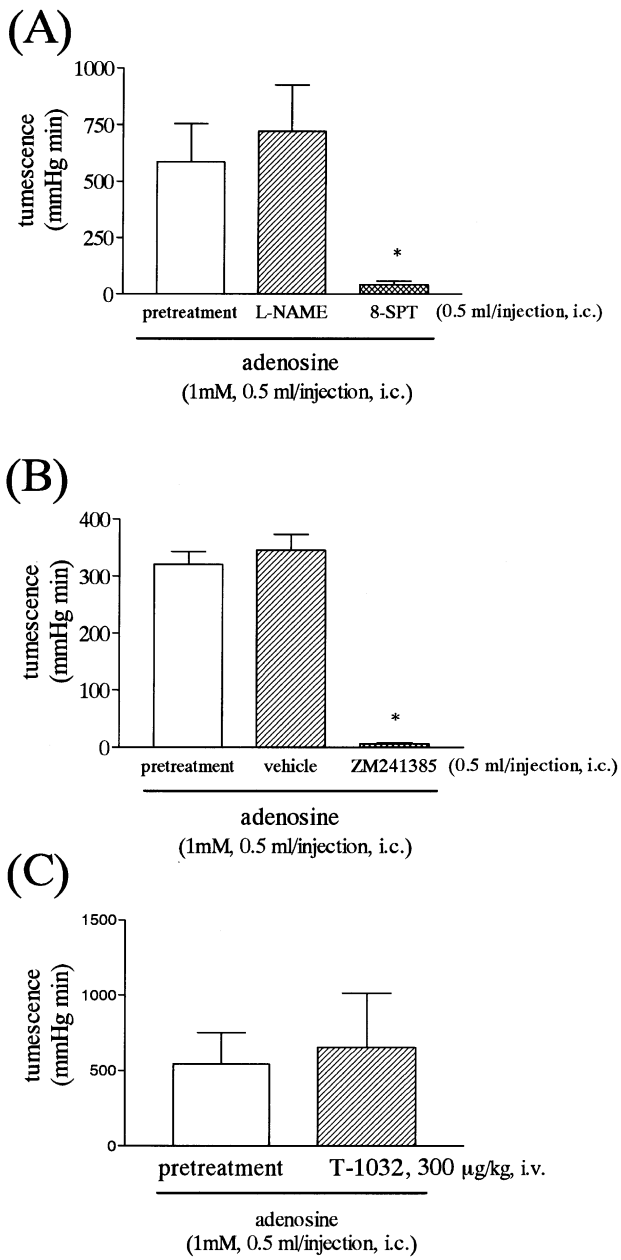

Fig. 2. (A) Effects of L-NAME (30 mM, 5 min before adenosine) and 8-SPT (1 mM, 5 min before adenosine) on the tumescence induced by adenosine (1 mM, i.c.) in anesthetized dogs ($n = 5$), $^*P < 0.05$. (B) Effects of ZM241385 (1 mM, i.c., 5 min before adenosine) on the tumescence induced by adenosine (1 mM, i.c.) in anesthetized dogs ($n = 5$), $^*P < 0.05$. (C) Effect of T-1032 (300 μg/kg, i.v., 5 min before adenosine) on the adenosine-induced tumescence in anesthetized dogs ($n = 3$).

using Prism (GraphPad Software, San Diego, USA). Differences were considered significant when $P < 0.05$.

2.3. Chemicals

Methyl 2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate (T-1032 synthesized at Tanabe Seiyaku, Saitama, Japan) was dissolved in saline containing 0.0025 N HCl. N^G-nitro-L-arginine, N^G-nitro-D-arginine (L-NAME, D-NAME, Sigma, St. Louis, MO), adenosine, ATP, ADP$_\beta$S (Sigma), 8-(*p*-sulfophenyl)theophylline (8-SPT, Sigma), reactive blue-2 (Sigma) and β, γ-meATP (Nacalai Tesque, Kyoto, Japan) were dissolved in saline. 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)phenol (ZM241385) (Nacalai Tesque) was dissolved in 1% dimethyl sulfoxide.

3. Results

Intracavernous adenosine injection (0.1, 0.3, and 1 mM, 0.5 ml/injection) induced tumescence dose-dependently (Fig. 1A). This tumescence could be induced repeatedly to the same degree at least three times (1 mM, 0.5 ml/injection, i.c.) (Fig. 1B) and was abolished by pretreatment with 8-SPT (1 mM, 0.5 ml/injection, i.c.), an unspecific adenosine receptor antagonist, and with ZM241385 (1 mM, 0.5 ml/injection, i.c.), a specific adenosine A_{2A} receptor antagonist (Fig. 2A,B). L-NAME (30 mM, 0.5 ml/injection, i.c.), an NO synthase inhibitor, and T-1032 (300 μg/kg, i.v.), a phosphodiesterase type V inhibitor, had no effect on the adenosine-induced tumescence (Fig. 2A,C). Intracavernous ATP injection (1, 2 and 3 mM, 0.5 ml/injection) also induced tumescence dose-dependently and this could be repeated at least three times, as with adenosine (Fig. 3). ATP-induced tumescence was inhibited by 8-SPT (1 mM, 0.5 ml/injection, i.c.) but not by reactive blue-2, a P2 receptor antagonist (10 mM, 0.5 ml/injection, i.c.) (Fig. 4A). Neither β, γ-meATP (3 mM, 0.5 ml/injection, i.c.), a P2X receptor agonist, nor ADP$_\beta$S (1 mM, 0.5 ml/injection, i.c.), a P2Y receptor agonist induced tumes-

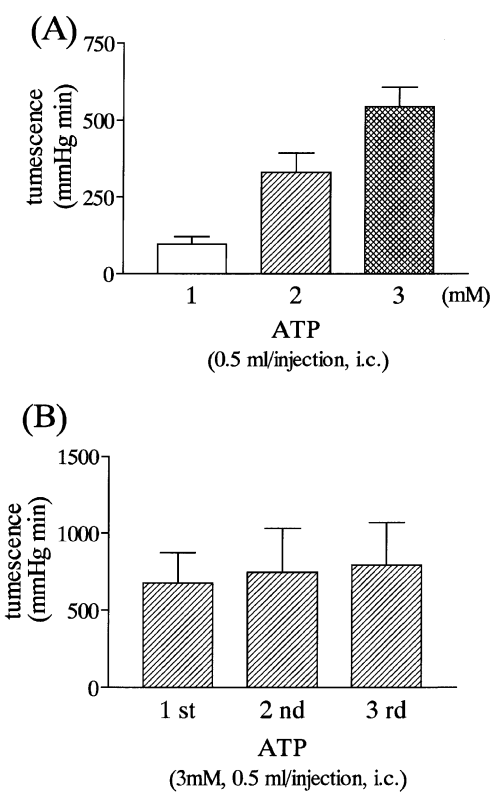

Fig. 3. (A) Intracavernous ATP injection (0.5 ml/injection) induced tumescence dose-dependently in anesthetized dogs ($n = 6$). (B) Repeated ATP (3 mM, i.c.) induced tumescence to the same extent ($n = 3$).

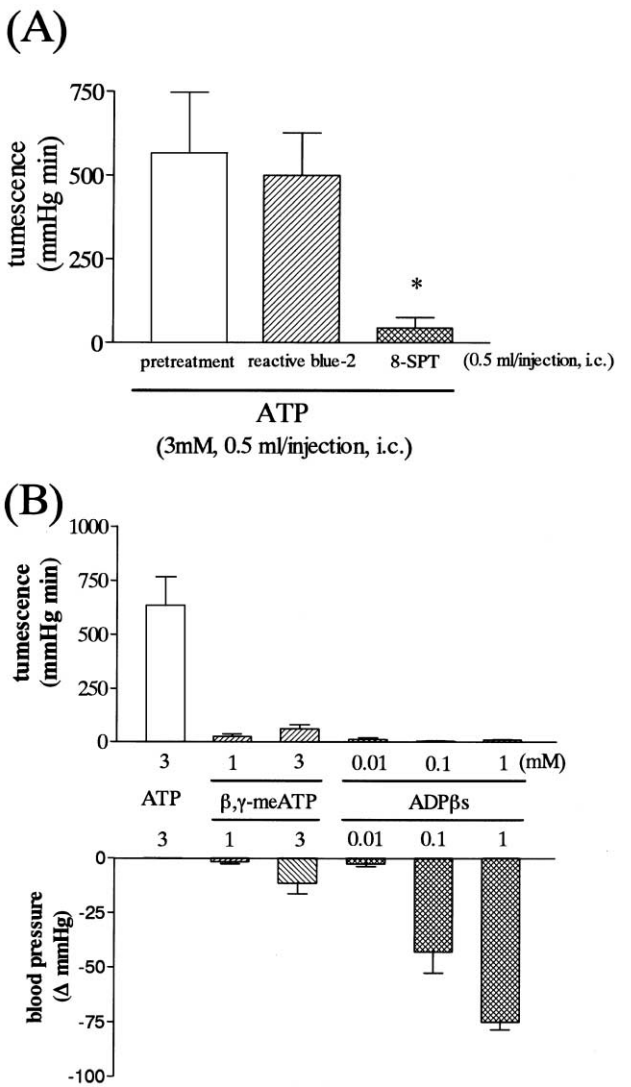

Fig. 4. (A) Effects of reactive blue-2 (10 mM, 5 min before ATP) and 8-SPT (1 mM, 5 min before ATP) on the tumescence induced by ATP (3 mM, i.c.) in anesthetized dogs ($n = 5$), $^*P < 0.05$. (B) Intracavernous β, γ-meATP (0.5 ml/injection) and ADP$_\beta$S (0.5 ml/injection) did not induce tumescence but lowered systemic blood pressure in anesthetized dogs ($n = 5$).

cence (Fig. 4B). These local injections of P2 receptor agonists lowered the systemic blood pressure, but none of the other treatments, including pelvic nerve stimulation, had any effect on this (Fig. 4B). The pelvic nerve stimulation-induced tumescence was inhibited by L-NAME (30 mM, 0.5 ml/injection, i.c.) but not by D-NAME (Fig. 5B). 8-SPT and reactive blue-2 had no effect on the tumescence induced by the pelvic nerve stimulation (Fig. 5C).

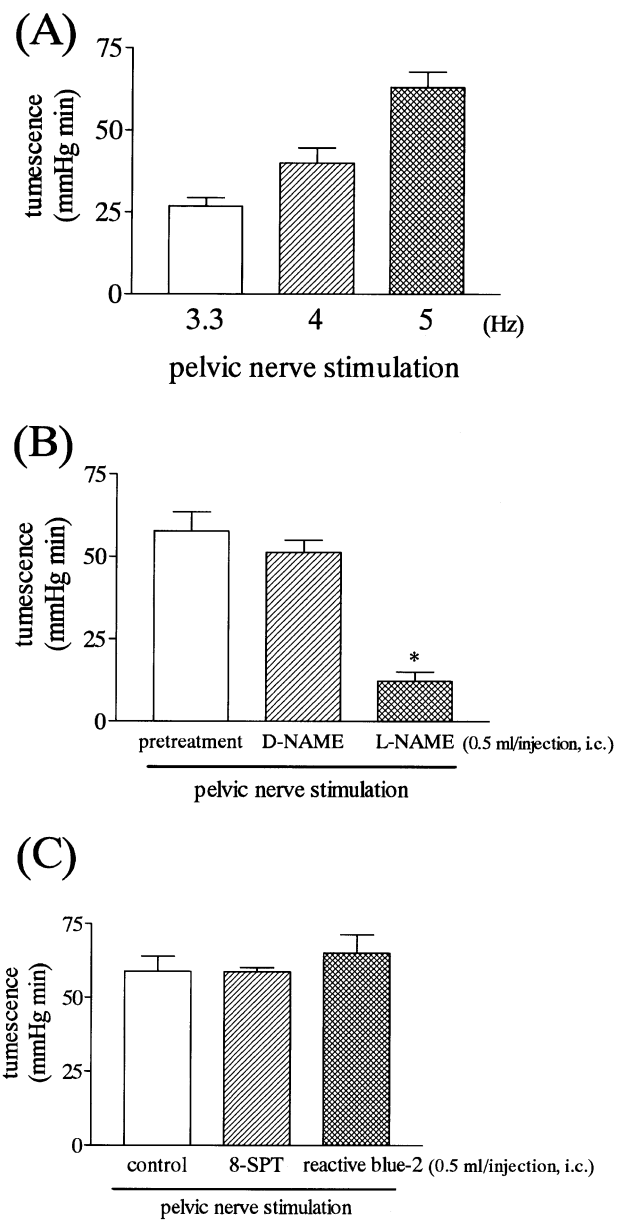

Fig. 5. (A) Pelvic nerve stimulation (200-μs pulse width, 10 V, 40-s duration) induced tumescence hertz dependently in anesthetized dogs ($n = 5$). (B) L-NAME (30 mM, 5 min before nerve stimulation) but D-NAME (30 mM, 5 min before nerve stimulation) inhibited the tumescence induced by pelvic nerve stimulation (5 Hz, 200-μs pulse width, 10 V, 40-s duration) in anesthetized dogs ($n = 5$), $^{*}P < 0.05$. (C) Effects of 8-SPT (1 mM, 5 min before nerve stimulation) and reactive blue-2 (10 mM, 5 min before nerve stimulation) on the tumescence induced by pelvic nerve stimulation (5 Hz, 200-μs pulse width, 10 V, 40-s duration) in anesthetized dogs ($n = 5$).

4. Discussion

Extensive reduction by L-NAME and potentiation by the phosphodiesterase type V inhibitor have suggested an essential role of NO in the pelvic nerve-stimulated tumescence in anesthetized dogs (Noto et al., 2000; Trigo-Rocha et al., 1993). Although intracavernous injections of adenosine and ATP were reported to induce tumescence in dogs (Takahashi et al., 1992a,b), their roles in the tumescence induced by pelvic nerve stimulation were not clear (Andersson and Wagner, 1995). The desensitization of adenosine and P2 receptors occurred in canine tissues (Moser et al., 1989; Palmer et al., 1994; Werner et al., 1996). Almost the same degree of responses to each repeated treatment with either adenosine or ATP in this study prompted us to reexamine the effects of some antagonists on the tumescence.

The lack of inhibitory effect of L-NAME on the relaxation of isolated rabbit corpus cavernosum indicates that adenosine acts through an NO-independent pathway in vitro (Gur and Ozturk, 2000; Mantelli et al., 1995). In the present study, the tumescence induced by the intracavernous adenosine was not influenced by either L-NAME (30 mM) or T-1032 (300 μg/kg). Because the lower dose of T-1032 (30 μg/kg i.v. bolus followed by 1 μg/kg/h i.v. infusion) was reported to potentiate the tumescence induced by exogenously applied sodium nitroprusside, an NO generator, NO is unlikely to participate in adenosine-induced tumescence (Noto et al., 2000). We suppose that adenylate cyclase/cAMP systems may contribute to this tumescence as reported for other tissues (Londos et al., 1980; Van Calker et al., 1978).

The adenosine-induced relaxation of isolated rabbit cavernosum was antagonized by the adenosine receptor antagonist in vitro (Mantelli et al., 1995). In this in vivo study, the tumescence induced by adenosine was abolished by 8-SPT, an unspecific adenosine receptor antagonist. Mantelli et al. (1995) also reported that the adenosine A_{2A} receptor was involved in the relaxation caused by adenosine because 2-[*p*-(carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680), an adenosine A_{2A} receptor agonist, strongly relaxed the specimen. However, Chiang et al. (1994) reported that CGS21680 had no effect on the relaxation. So, the receptor subtypes involved in this tumescence were further characterized by using ZM241385, a specific adenosine A_{2A} receptor antagonist (Lopes et al., 1999; Poucher et al., 1995). Extensive reduction by ZM241385 has suggested that adenosine A_{2A} receptors contributed to the adenosine-induced tumescence in this study.

Filippi et al. (1999) reported that, in isolated rabbit cavernosum, the relaxation caused by ATP was due to direct stimulation of P2 receptors different from the classical P2X and P2Y receptor subtypes, and not blocked by reactive blue-2. In the present in vivo study, the tumescence induced by ATP was also not influenced by reactive

blue-2, but we found that the pretreatment with 8-SPT totally abolished the ATP-induced tumescence in anesthetized dogs. As ATP is known to be metabolized to adenosine by nucleotidases in vivo (Kennedy et al., 1997), adenosine seems to be responsible for the tumescence induced by ATP in our study.

Whereas either β, γ-meATP, a stable P2X receptor agonist, or $ADP_{\beta}S$, a stable P2Y receptor agonist, is reported to relax the isolated cavernous smooth muscle in vitro (Shalev et al., 1999; Wu et al., 1993), we now found that intracavernous injection of both agonists lacked the ability to induce tumescence in vivo. This is in agreement with the above observation that P2 receptors are not involved in the tumescence induced by ATP. The lowering of systemic blood pressure by the local injection of both agonists excludes the possibility that the doses were too low to activate the P2X and P2Y receptors in vivo, respectively.

A previous report suggested that the inhibition by DMPX, an adenosine A_2 receptor antagonist, made it likely that adenosine receptors are involved in the field stimulation-induced relaxation of isolated corpus cavernosum in vitro (Chiang et al., 1994). In the present study, L-NAME strongly inhibited the tumescence induced by pelvic nerve stimulation, but had no effect on the adenosine-induced tumescence. In addition, the pelvic nerve stimulation-induced tumescence was not inhibited by 8-SPT. Because this dose of 8-SPT abolished the tumescence induced by exogenously applied adenosine, the adenosine receptor does not seem to participate in the tumescence induced by pelvic nerve stimulation in vivo.

The lack of any effects of ATP and β, γ-meATP has suggested that the field stimulation-induced relaxation of isolated cavernous smooth muscle does not involve the P2 receptors in vitro (Wu et al., 1993). In the present study, reactive blue-2 showed no effect on the tumescence induced by pelvic nerve stimulation. In addition, intracavernous injection of either P2X or P2Y receptor agonist did not induce tumescence. In agreement with results obtained with isolated cavernous smooth muscles, the present study showed that P2 receptors have no role in the tumescence induced by pelvic nerve stimulation.

In summary, the present study showed that (1) exogenous adenosine induces tumescence, acting through the adenosine A_{2A} receptor, (2) adenosine is responsible for the ATP-induced tumescence, (3) neither adenosine nor P2 receptor is involved in the tumescence induced by pelvic nerve stimulation in anesthetized dogs.

References

Anderson, K.E., 1993. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol. Rev. 45, 253–308.

Andersson, K.E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75, 191–236.

Chiang, P.H., Wu, S.N., Tsai, E.M., Wu, C.C., Shen, M.R., Huang, C.H., Chiang, C.P., 1994. Adenosine modulation of neurotransmission in penile erection. Br. J. Clin. Pharmacol. 38, 357–362.

Filippi, S., Amerini, S., Maggi, M., Natali, A., Ledda, F., 1999. Studies on the mechanisms involved in the ATP-induced relaxation in human and rabbit corpus cavernosum. J. Urol. 161, 326–331.

Gur, S., Ozturk, B., 2000. Altered relaxant responses to adenosine and adenosine 5′-triphosphate in the corpus cavernosum from men and rats with diabetes. Pharmacology 60, 105–112.

Ignarro, L.J., Bush, P.A., Buga, G.M., Wood, K.S., Fukuto, J.M., Rajfer, J., 1990. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem. Biophys. Res. Commun. 170, 843–850.

Kennedy, C., Todorov, L.D., Mihaylova-Todorova, S., Sneddon, P., 1997. Release of soluble nucleotidases: a novel mechanism for neurotransmitter inactivation? Trends Pharmacol. Sci. 18, 263–266.

Londos, C., Cooper, D.M., Wolff, J., 1980. Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. U. S. A. 77, 2551–2554.

Lopes, L.V., Cunha, R.A., Ribeiro, J.A., 1999. ZM 241385, an adenosine A(2a) receptor antagonist, inhibits hippocampal A(1) receptor responses. Eur. J. Pharmacol. 383, 395–398.

Mantelli, L., Amerini, S., Ledda, F., Forti, G., Maggi, M., 1995. The potent relaxant effect of adenosine in rabbit corpora cavernosa is nitric oxide independent and mediated by A_2 receptors. J. Androl. 16, 312–317.

Moser, G.H., Schrader, J., Deussen, A., 1989. Turnover of adenosine in plasma of human and dog blood. Am. J. Physiol. 256, C799–C806.

Noto, T., Inoue, H., Ikeo, T., Kikkawa, K., 2000. Potentiation of penile tumescence by T-1032: a new potent and specific phosphodiesterase type V inhibitor, in dogs. J. Pharmacol. Exp. Ther. 294, 870–875.

Palmer, T.M., Gettys, T.W., Jacobson, K.A., Stiles, G.L., 1994. Desensitization of the canine A_{2A} adenosine receptor: delineation of multiple processes. Mol. Pharmacol. 45, 1082–1094.

Poucher, S.M., Keddie, J.R., Singh, P., Stoggall, S.M., Caulkett, P.W., Jones, G., Coll, M.G., 1995. The in vitro pharmacology of ZM 241385: a potent, non-xanthine A_{2A} selective adenosine receptor antagonist. Br. J. Pharmacol. 115, 1096–1102.

Ragazzi, E., Chinellato, A., Italiano, G., Pagano, F., Calabro, A., 1996. Characterization of in vitro relaxant mechanisms in erectile tissue from rabbits of different ages. Urol. Res. 24, 317–322.

Shalev, M., Staerman, F., Allain, H., Lobel, B., Saiag, B., 1999. Stimulation of P2Y purinoceptors induces, via nitric oxide production, endothelium-dependent relaxation of human isolated corpus cavernosum. J. Urol. 161, 955–959.

Takahashi, Y., Ishii, N., Lue, T.F., Tanagho, E.A., 1992a. Effects of adenosine on canine penile erection. J. Urol. 148, 1323–1325.

Takahashi, Y., Ishii, N., Lue, T.F., Tanagho, E.A., 1992b. Effects of adenosine triphosphate on canine penile erection. Int. J. Impot. Res. 4, 27–34.

Trigo-Rocha, F., Aronson, W.J., Hohenfellner, M., Ignarro, L.J., Rajfer, J., Lue, T.F., 1993. Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. Am. J. Physiol. 264, H419–H422.

Van Calker, D., Muller, M., Hamprecht, B., 1978. Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. Nature 276, 839–841.

Werner, P., Seward, E.P., Buell, G.N., North, R.A., 1996. Domains of P2X receptors involved in desensitization. Proc. Natl. Acad. Sci. U. S. A. 93, 15485–15490.

Wu, H.Y., Broderick, G.A., Suh, J.K., Hypolite, J.A., Levin, R.M., 1993. Effects of purines on rabbit corpus cavernosum contractile activity. Int. J. Impot. Res. 5, 161–167.