J. Pharm. Pharmacol. 1996, 48: 96–99
Received June 12, 1995
Accepted September 12, 1995

Involvement of σ-Receptors in the Increase in Contraction of Mouse Vas Deferens Induced by Exogenous ATP

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Abstract

The effects of σ-receptor ligands on the twitch contraction elicited by the exogenous application of adenosine 5′-triphosphate (ATP) in the unstimulated mouse vas deferens were studied.

(−)-Pentazocine, 1,3-di(2-tolyl)guanidine (DTG) and two pairs of optical isomers of 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine (3-PPP) and *N*-allylnormetazocine (SKF-10,047) potentiated the exogenous application of ATP-induced twitch-type contraction in a concentration-dependent manner, while (+)-pentazocine did not affect it. The order of potentiating ability was: (+)-3-PPP > (−)-pentazocine > (−)-SKF-10,047 > DTG > (−)-3-PPP > (+)-SKF-10,047. On the other hand, haloperidol and rimcazole, putative σ-receptor antagonists, suppressed this twitch contraction. In addition, these antagonists significantly blocked the (+)-3-PPP- and (−)-pentazocine-induced potentiation at concentrations which did not affect contractions per se.

These findings indicate that the exogenous application of ATP-induced twitch contraction in the mouse vas deferens is regulated by σ-receptors. In addition, the present ranking order suggests that the σ-receptor potentiating the ATP-induced twitch contraction at post-junctional sites may differ from the σ_1- and/or σ_2-receptor subtypes.

Since the identification of the σ-receptor was reported (Quirion et al 1987), intensive studies to elucidate the physiological function of the σ-receptor have been carried out (Walker et al 1990; Ferris et al 1991; Su 1991). In the rodent isolated vas deferens, σ-receptor ligands are reported to potentiate the electrically evoked neurogenic twitch contraction (Campbell et al 1987; Vaupel & Su 1987, 1988; Kennedy & Henderson 1989; Fox et al 1991). However, whether the σ-receptor ligands-induced potentiations are mediated through σ-receptors in these tissues has been questioned. Recently, we have demonstrated that the potentiations of neurogenic twitch contraction elicited by σ-receptor ligands are mediated through the benzomorphan-type σ-receptor in the mouse vas deferens (Matsuno et al 1993). Briefly, the order of potency of σ-receptor ligands to potentiate the neurogenic twitch contraction was significantly correlated with the potency to inhibit [^{3}H](+)-*N*-allylnormetazocine ([^{3}H](+)-SKF-10,047) binding in the mouse vas deferens.

Neurogenic twitch contraction of the mouse vas deferens is reportedly mediated by the released adenosine 5′-triphosphate (ATP), but not by noradrenaline, in DBA/1A (Kennedy & Henderson 1989), Swiss albino (Rae & Calixto 1989) and ddY mice (Seong et al 1990). Similarly, we have shown that only ATP is released by nerve stimulation, and elicits a twitch-type contractile response in isolated ddY mouse vas deferens (Matsuno & Mita 1992). Thus, it is possible that the σ-receptor ligand-induced potentiation of the neurogenic twitch contraction in this preparation is caused by an enhancement of ATP transmission at the pre- or post-junctional sites.

In the present study, we examined whether σ-receptor ligands regulate the exogenous application of ATP-induced twitch-type contraction at the post-junctional sites in unstimulated ddY mouse vas deferens.

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Materials and Methods

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the 'Guide for the Care and Use of Laboratory Animals' (NIH publication, No. 85-23 1985).

Isolated mouse vas deferens

Experiments using mouse vas deferens were performed as described previously (Matsuno & Mita 1992). Briefly, male ddY mice (Nihon SLC, Shizuoka, Japan), 35 to 50 g, were killed by decapitation. Their vasa deferentia were dissected out, cleaned and mounted in 20-mL organ baths at a resting tension of 200 mg. The tissues were bathed in a modified Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4·75, $CaCl_2$ 2·54, KH_2PO_4 1·2, $NaHCO_3$ 25 and D-glucose 11. The solution was maintained at 37°C and bubbled with 95% O_2–5% CO_2. Naloxone (1 μM) was present throughout the experiments to prevent inhibitory actions mediated by opioid receptors. Contractions were evoked by the exogenous application of ATP (1 mM) and were reproducible for more than 5 h. The time interval between applications of ATP was at least 30 min. Contractions were measured isometrically with a force-displacement transducer (Nihon Kohden TB-612T).

Assessment of σ-receptor ligands

The potencies of σ-receptor ligands were determined by applying a single dose and measuring the twitch height once the response had finished. The drug was washed out by overflow each time and the response was reversible after washout of drugs. Concentration-response curves were

determined in each tissue. Changes in the twitch amplitude were calculated as a percentage of the control twitches measured immediately before drug addition. σ-Receptor ligands were added 5 min before the application of ATP (Seong et al 1990; Matsuno & Mita 1992). To clarify whether the potentiating effects of σ-receptor ligands were mediated via the σ-receptor, we attempted to block these potentiating effects by using haloperidol and rimcazole, putative σ-receptor antagonists. Each putative σ-receptor antagonist was applied 5 min before the addition of (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) or (−)-pentazocine (Vaupel & Su 1987; Matsuno et al 1993).

Data analysis

The results are expressed as the means ± s.e.m. Comparisons between two groups were analysed by using Student's unpaired *t*-test. *P* values of 0·05 or less were considered statistically significant. The concentration required to produce a 50% potentiation was calculated with a dose-response relationship using computer-assisted linear least-squares regression analysis.

Drugs

The following drugs were used: 1,3-di(2-tolyl)guanidine (DTG), (+)-3-PPP, (−)-3-PPP, (+)-SKF-10,047, (−)-SKF-10,047 and rimcazole (Research Biochemicals Incorporated); haloperidol (Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan); naloxone hydrochloride and ATP (Sigma). (+)-Pentazocine and (−)-pentazocine were synthesized in the laboratories of Santen Pharmaceutical Co., Ltd. Other chemicals and reagents of analytical grade were obtained from commercial suppliers. Drugs were dissolved in distilled water, except DTG, which was initially dissolved in 1 M HCl and neutralized with 1 M NaOH.

Results

Effects of various σ-receptor ligands

(−)-Pentazocine and both optical isomers of SKF-10,047 concentration-dependently potentiated the exogenous application of ATP elicited twitch-type contraction of the unstimulated ddY mouse vas deferens, while (+)-pentazocine had no detectable effect on this response (Fig. 1). Similarly, DTG and both optical isomers of 3-PPP concentration-dependently potentiated the twitch contraction elicited by the exogenous application of ATP (Fig. 2). The potentiation elicited by benzomorphan (−)enantiomers was greater than that elicited by benzomorphan (+)enantiomers (Fig. 1), while the potentiation elicited by (+)-3-PPP was more potent than that elicited by (−)-3-PPP (Fig. 2). On the other hand, haloperidol and rimcazole, putative σ-receptor antagonists, concentration-dependently suppressed the exogenous application of ATP-induced twitch contraction of the mouse vas deferens (Fig. 2). σ-Receptor ligands did not cause any distinct change of basal tension of the unstimulated preparations at the concentration ranges used in the present study.

The concentrations causing a 50% potentiation were (μM): (+)-3-PPP 5·7, (−)-pentazocine 18·6, (−)-SKF-10,047 19·8, DTG 39·8, (−)-3-PPP 42·2 and (+)-SKF-10,047 112·3. The order of potentiation ability was: (+)-3-PPP > (−)-pentazocine > (−)-SKF-10,047 > DTG > (−)-3-PPP > (+)-SKF-10,047.

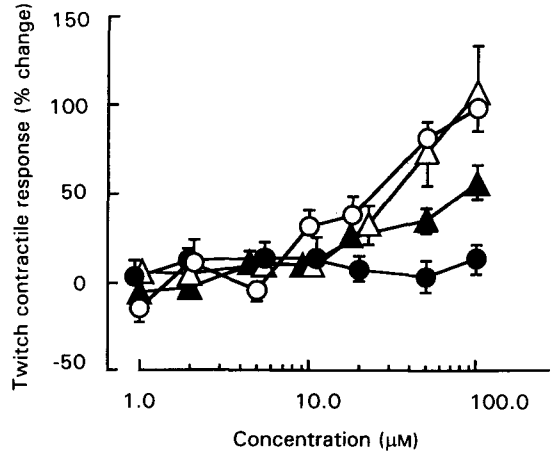

FIG. 1. Potentiation of the exogenous application of ATP-induced twitch contractions of the unstimulated mouse vas deferens elicited by stereoisomers of pentazocine and SKF-10,047. Amplitudes of the twitch contraction just before the addition of each drug were taken as 100%. The results are expressed as the means ± s.e.m. ○ (−)-pentazocine (n = 7); ● (+)-pentazocine (n = 7); △ (−)-SKF-10,047 (n = 8); ▲ (+)-SKF-10,047 (n = 8).

Effects of prior exposure to putative σ-receptor antagonists on (+)-3-PPP and (−)-pentazocine-induced potentiation

Haloperidol at concentrations of 1–5 μM, concentration-dependently blocked the (+)-3-PPP- and (−)-pentazocine-induced potentiation of the exogenous application of ATP-induced twitch contraction (Table 1). The significant antagonisms for (+)-3-PPP- and (−)-pentazocine-induced potentiation by haloperidol were observed at 1–5 μM, and 5 μM, respectively (Table 1). Similarly, rimcazole significantly blocked the (+)-3-PPP- and (−)-pentazocine-induced potentiation of the exogenous application of ATP-induced twitch contraction at 2 and 5 μM, and 5 μM, respectively (Table 1). Rimcazole, at 2 μM, appeared to augment the

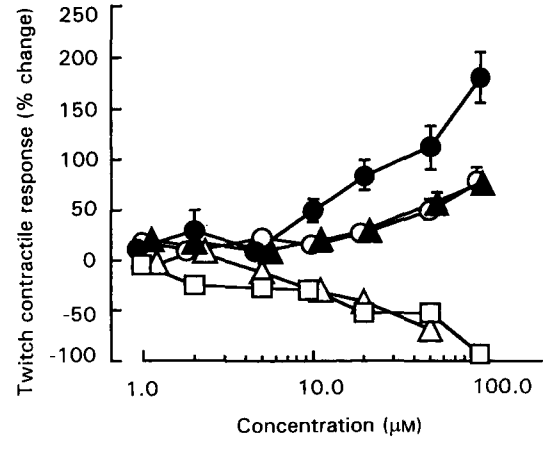

FIG. 2. Concentration-response curves of various σ-receptor ligands on the exogenous application of ATP-elicited twitch contraction of the unstimulated mouse vas deferens. Amplitudes of the twitch contraction just before the addition of each drug were taken as 100%. The results are expressed as the means ± s.e.m. ● (+)-3-PPP (n = 4–8); ○ (−)-3-PPP (n = 8); ▲ DTG (n = 8); △ haloperidol (n = 8); □ rimcazole (n = 8).

Table 1. Antagonism by haloperidol and rimcazole of the induced potentiation of the exogenous application of ATP-elicited twitch contraction of mouse vas deferens.

Inducer (50 μM)	Antagonist	Concn (μM)	Twitch contraction (% control)
(+)-3-PPP	Haloperidol	–	207 ± 18**
		1	152 ± 17*+
		2	140 ± 13*++
		5	100 ± 11++
	Rimcazole	–	190 ± 19**
		1	186 ± 21**
		2	137 ± 16*+
		5	128 ± 17+
(−)-Pentazocine	Haloperidol	–	176 ± 18**
		1	160 ± 17**
		2	177 ± 20**
		5	83 ± 12++
	Rimcazole	–	162 ± 7**
		1	160 ± 10**
		2	204 ± 20**
		5	134 ± 10*+

Means ± s.e.m. from 8–10 preparations. *$P<0.05$, **$P<0.01$ compared with control. +$P<0.05$, ++$P<0.01$ compared with inducer alone.

(−)-pentazocine-induced potentiation of the exogenous application of ATP-induced twitch contraction (Table 1).

Discussion

The exogenous application of ATP-induced twitch contractions in the unstimulated mouse vas deferens were potentiated by putative σ-receptor agonists. In addition, the putative σ-receptor agonist-induced augmentation was blocked by pretreatment with putative σ-receptor antagonists. These findings demonstrate that the exogenous application of ATP-induced twitch contraction in this tissue was regulated via a σ-receptor.

Since the classifications of σ-receptor subtypes have been proposed, there appear to exist at least two subtypes of the σ-receptor termed σ_1 and σ_2. The σ_1-receptors are characterized by stereoselectivity to the benzomorphan (+)enantiomer, while the σ_2-receptors are more sensitive to the benzomorphan (−)enantiomer (Hellewell & Bowen 1990; Su et al 1991; Quirion et al 1992). In the present study, the abilities of (−)-pentazocine and (−)-SKF-10,047 in potentiating the exogenous application of ATP-induced twitch contraction were more potent than the respective (+)enantiomer. Therefore, the stereoselectivity of benzomorphan in the present study suggests that the σ-receptor regulating the exogenous application of ATP-induced twitch contraction in the mouse vas deferens belongs to the σ_2-receptor subtype. On the other hand, the rank order of σ-receptor ligands to inhibit σ_2-receptor binding in the rat brain, heart, liver, or pheochromocytoma (PC12) cells was reported to be DTG = haloperidol > (−)-pentazocine = (+)-3-PPP > (+)-pentazocine = (−)-3-PPP > (−)-SKF-10,047 > (+)-SKF-10,047 (Dumont & Lemair 1991; DeHaven-Hudkins et al 1994; Hellewell et al 1994; McCann et al 1994). However, this rank order is not in agreement with the present rank order of σ-receptor ligands to potentiate the exogenous application of ATP-induced twitch contraction ((+)-3-PPP > (−)-pentazocine > (−)-SKF-10,047 > DTG > (−)-3-PPP > (+)-SKF-10,047). In particular, although DTG has been reported to have the highest affinity for the σ_2-receptor in several tissues among this series of σ-ligands (Dumont & Lemaire 1991; DeHaven-Hudkins et al 1994; Hellewell et al 1994; McCann et al 1994), the present ranking order of DTG is intermediate, and DTG is less potent than (+)-3-PPP, (−)-pentazocine or (−)-SKF-10,047. Therefore, the present results suggest that the σ-receptor regulating the exogenous application of ATP-induced twitch contraction in mouse isolated vas deferens differs from the σ_1- and/or σ_2-receptor subtypes. Recently, computer-assisted analysis in a displacement study proposed that the σ-receptor consisted of four different binding sites, R_1, R_2, R_3 and R_4 (Zhou & Musacchio 1991). The R_1 site exhibits high affinity for dextromethorphan, (+)-3-PPP and DTG. The R_2 site exhibits high affinity for dextromethorphan and low affinity for (+)-3-PPP. The R_3 site shows high affinity for DTG, intermediate affinity for (+)-3-PPP and low affinity for dextromethorphan. Finally, the R_4 site has intermediate affinity for (+)-3-PPP and low affinity for DTG and dextromethorphan. The R_1 and R_3 sites were consistent with the σ_1- and σ_2-receptor, respectively. The R_2 and R_4 sites were consistent with the dextromethorphan-selective site and the low-affinity site, respectively (Zhou & Musacchio 1991). Taking this classification into consideration, the σ-receptor regulating the exogenous application of ATP-induced twitch contraction in the mouse vas deferens may be the R_4 site (the low-affinity site of the σ-receptor). In fact, we previously reported that the mouse vas deferens possessed the high and low affinity sites of the [^{3}H](+)-SKF-10,047 binding sites (Matsuno et al 1993). Thus, it is possible that the R_4 site belongs to the low-affinity site of [^{3}H](+)-SKF-10,047 binding sites. Although the low-affinity site of [^{3}H](+)-SKF-10,047 binding sites was reported to be the *N*-methyl-D-aspartate (NMDA) receptor-channel complex (Largent et al 1986), our previous report has shown that (+)-5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclo-hepten-5,10-imine ((+)-MK-801), the non-competitive antagonist of NMDA receptor-channel complex, had no detectable effect on the neurogenic twitch contraction in this tissue (Matsuno et al 1993).

The ATP-induced twitch contraction was reportedly mediated through the post-junctional P_{2x} purinoceptors, because the stable ATP analogue, α,β-methylene ATP, an agent known to stimulate and then to desensitize P_{2x} purinoceptors, caused a concentration-dependent inhibition of the twitch response in the mouse vas deferens (Stjärne & Åstrand 1985; Allcorn et al 1986; Seong et al 1990; Matsuno & Mita 1992). In addition, the present study shows that the regulation of exogenous application of ATP-induced twitch contraction might be mediated through the R_4 site. Thus, the R_4 site of the σ-receptor may regulate the activity of the post-junctional P_{2x} purinoceptor in the vas deferens smooth muscle.

In conclusion, the present study shows that σ-receptors are involved in the exogenous application of ATP-induced twitch contraction in the mouse vas deferens. In addition, the present ranking order suggests that the σ-receptor potentiating the ATP transmission at post-junctional sites differs from the σ_1- and σ_2-receptor subtypes.

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