Evidence for Sympathetic, Purinergic Transmission in the Iris Dilator Muscle of the Rabbit

Ikunobu Muramatsu, Shigeru Kigoshi and Yasuo Oda

Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan
Faculty of Pharmaceutical Sciences, Kinki University, Higashi-Osaka 577, Japan

Received February 28, 1994 Accepted June 25, 1994

ABSTRACT—Electrical transmural stimulation of isolated iris dilator muscle of the rabbit produced a transient contraction that consisted of adrenergic and nonadrenergic components. In contrast to the adrenergic component, the nonadrenergic component was resistant to prazosin and other adrenoceptor antagonists. However, both components were completely blocked by guanethidine or tetrodotoxin. Among some tested compounds including neuropeptide Y, both ATP and 2-methylthio ATP produced a transient contraction in the dilator muscle and the sustained treatment with each markedly attenuated the nonadrenergic responses to electrical stimulation and to ATP. Suramin had no effect on and \( \alpha, \beta \)-methylene ATP potentiated the responses to electrical stimulation and to ATP. These results suggest that the nonadrenergic contraction induced by electrical transmural stimulation is a sympathetic purinergic response that may be mediated through unique purinoceptors.

Keywords: Iris dilator muscle, Sympathetic nonadrenergic response, Purinergic transmission, Purinoceptor

Iris dilator muscles are innervated by sympathetic nerves and the muscle tone is predominantly regulated by adrenergic mechanisms (1–3). However, there is evidence that the sympathetic responses in some other tissues are also elicited by nonadrenergic mechanisms (4, 5) in addition to adrenergic ones. Recently, we found that the response of isolated rabbit iris dilator muscle to electrical transmural stimulation was not completely blocked by prazosin. Here we report that the prazosin-resistant contraction of the dilator muscle is sympathetic in origin but may be mediated through a unique purinergic mechanism.

MATERIALS AND METHODS

Albino rabbits of either sex, weighing 2 to 3 kg, were anesthetized with thiopentone sodium (30 mg/kg, i.v.), exanguinated from the common carotid arteries and then the eyes were enucleated. The iris dilator muscle strips, approximately 3 mm in width and 7 mm in length, were cut under a dissecting microscope, and the strip was mounted vertically in an organ bath containing 20 ml modified Krebs-Henseleit solution of the following composition: 112 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl\(_2\), 2 mM CaCl\(_2\), 25 mM NaHCO\(_3\), 1.2 mM Na\(_2\)HPO\(_4\) and 11.5 mM glucose. The bath medium was maintained at 37°C, pH 7.4 and was equilibrated with a gas mixture consisting of 95% O\(_2\) and 5% CO\(_2\). A resting tension of 0.1 g was applied, and the responses were recorded isometrically through force-displacement transducers. The preparations were allowed to equilibrate for 90 min in the bathing medium before the experiments were started. Electrical transmural stimulation was applied through a pair of platinum-wire electrodes. Stimulus parameters were 0.1 ms duration, a frequency of 10 or 20 Hz and supramaximum voltage (7.5 V) for 10 sec. Drugs were added directly to the bath. Experimental values are given as means±S.E. and were analyzed by Student’s t-test.

RESULTS

Electrical transmural stimulation of isolated iris dilator muscle of the rabbit caused a transient contraction. This response was abolished by guanethidine (5 \( \mu \)M) or tetrodotoxin (0.5 \( \mu \)M). Prazosin also inhibited but did not abolish the contractile response to electrical stimulation; thus approximately 23±5% (n=15) of the amplitude of the original response remained as a resistant component after treatment with 0.3 \( \mu \)M prazosin (Fig. 1). The
prazosin-resistant contraction was not inhibited by other adrenoceptor antagonists (1 μM WB4101, 1 μM rauwolscine, 1 μM propranolol) or a purinoceptor antagonist (100 μM suramin, Fig. 2A) (n = 3 for each drug). The dilator muscle did not respond to neuropeptide Y (0.1 μM), neurokinin A (0.1 μM), adenosine (100 μM), somatostatin (0.1 μM), histamine (100 μM) and serotonin (100 μM). However, the muscle produced a large contraction in response to ATP (EC₅₀ = 230 ± 34 μM, n = 6) (Fig. 1B).

The contractile response to ATP (1000 μM) was transient, and the sustained treatment with ATP produced marked reductions of the prazosin-resistant contraction to electrical stimulation and of the response to cumulatively applied ATP (Fig. 1B). However, ATP treatment did not attenuate the contractile response to noradrenaline (Fig. 3). 2-Methylthio ATP (10 μM) also produced a contraction and subsequently attenuated the prazosin-resistant response (Fig. 1C). On the other hand, α,β-methylene

---

**Fig. 1.** Effects of various drugs on the contractile responses of rabbit iris dilator muscles to electrical transmural stimulation (20 Hz, for 10 sec). Each drug was cumulatively applied. Prazosin (0.3 μM), guanethidine (5 μM), ATP (1000 μM), α,β-methylene ATP (α,β-Me ATP, 10 μM), 2-methylthio ATP (2-Me S ATP, 10 μM). Record C was obtained in the presence of 0.3 μM prazosin.

---

**Fig. 2.** Contractile responses to electrical transmural stimulation (20 Hz, for 10 sec) or ATP (300 μM) in the rabbit iris dilator muscles. A: effects of prazosin (0.3 μM), suramin (100 μM) and guanethidine (5 μM). B and C: effects of suramin (100 μM) and α,β-methylene ATP (10 μM) on ATP response, respectively.
ATP (10 µM) failed to cause a contraction but significantly (P<0.05) enhanced the prazosin-resistant response (33 ± 3% increase in amplitude, n=3) (Fig. 1C). The prazosin-resistant response to electrical stimulation and the response to ATP (300 µM) were not inhibited by suramin (100 µM, n=4 for each response) (Fig. 2, A and B). α,β-Methylene ATP (10 µM) significantly (P<0.05) potentiated the contractile response to ATP (24±3% increase, n = 3) (Fig. 2C).

DISCUSSION

Electrical transmural stimulation produced prazosin-sensitive and -resistant contractions in the rabbit iris dilator muscle. The prazosin-resistant contraction was also resistant to the other adrenoceptor antagonists tested. Since both the responses were abolished by guanethidine and tetrodotoxin, the resistant component was considered to be elicited by another substance that was released concomitantly with noradrenaline from sympathetic nerve terminals. Neuropeptide Y and ATP or a related nucleotide are now known to be cotransmitters in sympathetic nerve terminals. In fact, the sustained treatment with ATP or 2-methylthio ATP markedly attenuated not only the nonadrenergic contraction to electrical stimulation but also the response to cumulatively applied ATP.

It is interesting to note that the sympathetic purinergic responses observed in many peripheral tissues are predominantly mediated through P2X-purinoceptors and therefore that the responses are inhibited either by P2X-purinoceptor desensitization after its initial activation with α,β-methylene ATP or by a P2X, P2Y-purinoceptor antagonist, suramin (4, 5, 7). However, the rabbit iris dilator muscle did not respond to α,β-methylene ATP and rather contracted in response to a P2Y-purinoceptor agonist, 2-methylthio ATP (8). Furthermore, suramin showed no inhibitory effect on the nonadrenergic responses to electrical stimulation and to exogenous ATP, and α,β-methylene ATP did potentiate both the nonadrenergic responses. These results suggest that the sympathetic purinergic response of the rabbit iris dilator muscle is caused through a unique purinoceptor that may be different from the P2X and P2Y-subtypes in pharmacological features. This may be the first demonstration of purinergic transmission in the eye.

Acknowledgments

The authors thank Dr. K. Shinozuka (Shimane Medical University) for the kind gift of suramin and Mrs. N. Aoki for secretarial assistance. This work was supported in part by a grant from the Smoking Research Foundation in Japan.

REFERENCES

1 Richardson KC: The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles. Am J Anat 114, 173–205 (1964)
2 Takayanagi I, Shiraishi K and Satoh M: Effects of ageing on responses of rabbit iris smooth muscles to agonists and field stimulation. Gen Pharmacol 23, 463–469 (1992)
3 Yoshitomi T and Ito Y: Double reciprocal innervations in dog iris sphincter and dilator muscles. Invest Ophthalmol Vis Sci 27, 83–91 (1986)
4 Burnstock G: The changing face of autonomic neurotransmission: (The First von Euler Lecture in Physiology). Acta Physiol Scand 126, 67–91 (1986)
5 Kügelgen IV and Starke K: Noradrenaline-ATP co-transmission in the sympathetic nervous system. Trends Pharmacol Sci 12, 319–324 (1991)
6 Muramatsu I: Evidence for sympathetic, purinergic transmission in the mesenteric artery of the dog. Br J Pharmacol 87, 478–480 (1986)
7 Muramatsu I, Ohmura T and Oshita M: Comparison between sympathetic adrenergic and purinergic transmission in the dog mesenteric artery. J Physiol (Lond) 411, 227–243 (1989)
8 Burnstock G and Kennedy C: Is there a basis for distinguishing two types of P2-purinoceptor? Gen Pharmacol 16, 433–440 (1985)