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Inhibitory Effect of Papaverine on the Contraction Induced by Transmural Stimulation in the Isolated Mouse Vas Deferens

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Abstract □ The effect of papaverine on the contractions induced by adrenergic neurotransmission in the isolated mouse vas deferens was investigated. Papaverine, 10^{-7}-10^{-5}M, showed a dose-dependent and reversible inhibition on the induced contractions. When the frequency of stimulation was varied (2.5-20.0 Hz), the inhibitory effect tended to be marked at the lower frequencies.

Keywords □ Papaverine, mouse vas deferens, adrenergic neurotransmission, noradrenaline, acetylcholine

Papaverine, the main benzylisoquinoline alkaloid of opium, has a variety of effects on smooth muscle which result in relaxation and inhibition of responses to some excitatory agents[1]. Papaverine inhibits adenosine 3',5'-cyclic monophosphate (cAMP) phosphodiesterase and increase cytoplasmic cAMP concentration in smooth muscles[2,3]. Papaverine has been also reported to inhibit Ca^{2+} fluxes in depolarized guinea-pig taenia coli[4], rat vas deferens and urinary bladder[5]. D'Agostino *et al.*[6] reported the competitive action of papaverine on norepinephrine-induced contractile response in rabbit aorta. Recently, we reported that acetylcholinesterase activity of rat brain could be increased by papaverine and related compounds[7]. However, there has been no knowledge about actions of papaverine on adrenergic neurotransmission. The vas deferens of many animal species including mouse are richly innervated by adrenergic neurons and contain large amounts of noradrenaline, which is widely used as a model preparation for studying sympathetic neuroeffector transmission. The aim of the present investigation was to study the action of papaverine on adrenergic neurotransmission in the mouse isolated vas deferens.

EXPERIMENTAL METHODS

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Animals

Male mice (ICR Swiss) weighing 25-30g were used. They were housed in cages with free access to laboratory chows and water, and maintained in the environment of constant temperature and humidity throughout the experiments. They were killed by cervical dislocation.

Chemicals

Papaverine hydrochloride and (−)-noradrenaline bitartrate were purchased from Sigma Chemical Co. Acetylcholine was from Tokyo Kassei Co. Other chemicals used were of a first grade.

Preparation of vas deferens

Vas deferens was removed from a mouse and placed in Krebs-Ringer bicarbonate solution. It was carefully stripped of adhering fat, connective tissue and blood vessels. The isolated tissue was mounted under a resting tone of 0.3 to 0.4g in organ bath containing 15 ml Krebs-Ringer bicarbonate solution bubbled with 95% O_2 and 5% CO_2 at 36°C. The Krebs-Ringer bicarbonate solution contained 115.5 mM NaCl, 4.63 mM KCl, 2.47 mM $CaCl_2$, 1.16 mM $MgCl_2$, 1.16 mM NaH_2PO_4, 21.9 mM $NaHCO_3$, and 11 mM glucose.

Transmural stimulation

The preparation was allowed to equilibrate for

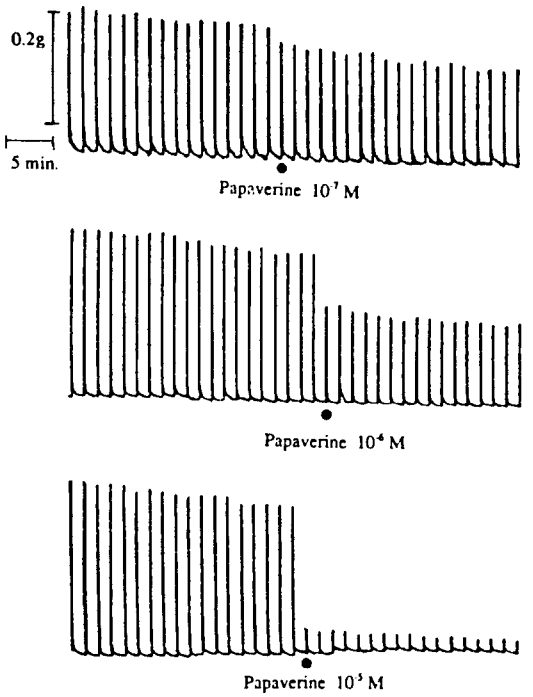

Fig. 1. Inhibitory patterns of papaverine on the mechanical responses of isolated mouse vas deferens by transmural stimulation (5 Hz, 1 ms, supramaximal voltage for 2 sec).

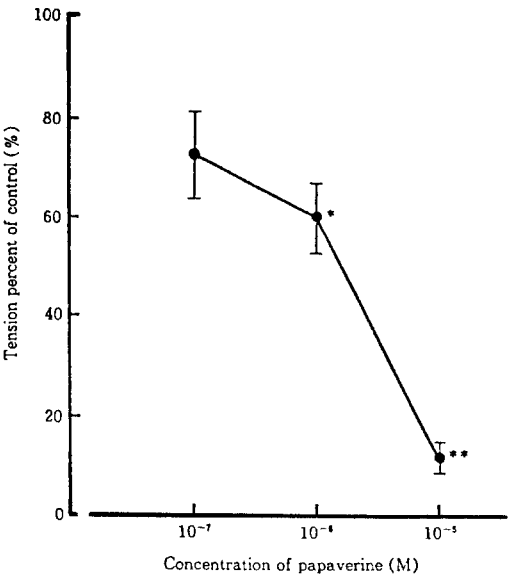

Fig. 2. Effect of papaverine on the mechanical responses of isolated mouse vas deferens by transmural stimulation (5 Hz, 1 ms, supramaximal voltage for 2 sec).

Values are given as a percentage of the control contraction before the addition of papaverine at each concentration. Each value was obtained from 8 preparations. Vertical bars represent mean± S.E.M.

* $p<0.05$, ** $p<0.01$.

60 min. Transmural stimulation (5 Hz, 1 msec, supramaximal voltage for 2 sec, unless otherwise indicated) was performed every 90 seconds with a square wave stimulator (Narco, SI-10) *via* a pair of electrodes according to the method described by Birmingham and Wilson[8]. Induced contractions were recorded on an ink-writing oscillograph (Narco, MK-IV) using an isometric force displacement transducer (Narco, F-60).

Measurement of papaverine effect

When the contractile response to transmural stimulation had become stable, the increasing concentrations of papaverine were added to the bath. The frequency-response curve for transmural stimulation was obtained by varying frequency of pulses from 2.5 to 20 Hz and stimulating the tissue every 90 sec until its contractile response reached a maximum as described by Day[9]. Drugs were added directly to the bath, and doses of each drug were expressed as final bath concentrations.

RESULTS AND DISCUSSION

Papaverine in the range of 10^{-7}-10^{-5}M inhibited the contractions induced by transmural stimulation in a dose-dependent manner, as shown in Fig. 1. The onset of its inhibitory effect was fairly rapid; maximal inhibition was obtained within 10-20 min and persisted over 30 min. Papaverine-induced inhibition on the response to transmural stimulation was reversible and papaverine had no effect on basal tension at the concentrations from 10^{-7}M to 10^{-3}M (data not shown). The relationship between dose and degree of inhibition was obtained from 8 different preparations for each concentration of papaverine (Fig. 2). Papaverine at 10^{-5}M significantly reduced the response to transmural stimulation to 11% of the control response.

For a given pulse duration and number of pulse, frequency was varied (2.5-20 Hz) to investigate the

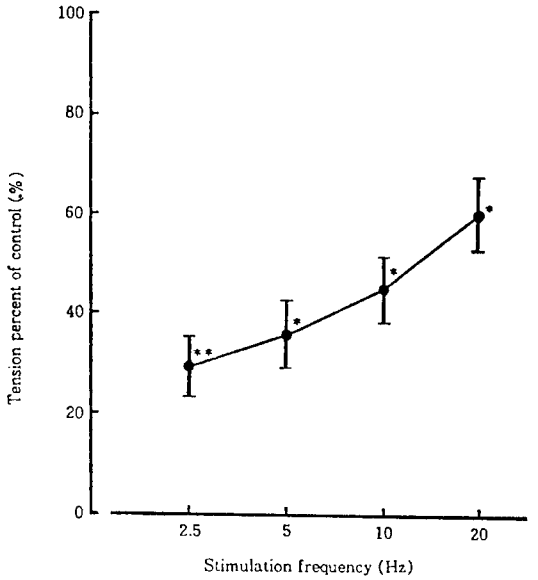

Fig. 3. Effect of papaverine (3×10^{-6}M) on the mechanical responses of isolated mouse vas deferens by transmural stimulation at various frequencies (2.5-20 Hz, 1 ms, supramaximal voltage for 2 sec). Each value was obtained from 8 preparations. Vertical bars represent mean± S.E.M. *p<0.05, **p<0.01

frequency dependence of papaverine-induced inhibition on the response to transmural stimulation. Papaverine at 3×10^{-6}M inhibited the response at all frequencies used for the stimulation and the inhibition tended to be marked at the lower frequencies of stimulation (Fig. 3). To evaluate whether papaverine in these concentrations can directly influence on the smooth muscle, we studied the effect of papaverine used together with two vas-contracting agonists. Experiments were carried out utilizing exogenous noradrenaline (10^{-5}M) and acetylcholine (10^{-5}M). Contractions caused by noradrenaline and acetylchloline were not affected by papaverine at 10^{-7}-10^{-6}M. However, papaverine at 10^{-5}M significantly reduced the contractile response of the vas deferens evoked by noradrenaline and acetylcholine about 30% and 31%, respectively (Table I).

Though the isolated rodent vas deferens has been frequently used to study on the drugs interfering with sympathetic transmission, the effect of papaverine on the contractile response to transmural stimulation has not been studied in this tissue. We have attempted to characterize the influence of papaverine on the twitch response of the transmural-stimulated mouse vas deferens. Recent studies have accumulated evidences that at least three components mediate the twitch response of the transmural-stimulated mouse vas deferens comprising a cholinergic component besides noradrenergic and purinergic components[10-14].

Table I. Effect of papaverine on noradrenaline (NA) and acetylcholine (ACh)-induced contractions

Papaverine	Contraction induced by NA (10^{-5}M)	Contraction induced by ACh (10^{-5}M)
Control	100(7)	100(7)
10^{-7}M	100.29± 2.45(7)	98.39± 2.41(7)
10^{-6}M	100.39± 4.08(7)	97.44± 5.81(7)
10^{-5}M	71.69± 7.25(7)*	69.13± 8.29(7)*

Drug-induced contractions in the presence of papaverine are expressed as a percentage of those in the absence of papaverine. The numbers in the parentheses indicate the number of experiments.
*p<0.05.

Papaverine inhibited the twitch contractions of isolated mouse vas deferens in response to transmural stimulation in a dose-dependent fashion, and the inhibition was inversely related to the frequency of stimulation. The finding that the degree of inhibition induced by papaverine was less striking at the higher frequencies might be related to an increase in Ca^{2+} influx per stimulation by increasing the frequency[15].

Since mechanical response to the transmural stimulation can be affected by both pre- and post-junctional actions of a drug, we tried to investigate the effect of papaverine on contractions evoked by exogenously-applied agonists acting directly on the smooth muscle. From the results (Fig. 2, Table I), high concentrations of papaverine, 10^{-5}M, greatly inhibited the contractile responses caused by transmural stimulation, 89%, whereas it moderately inhibited the contractions due to exogenous noradrenaline, 30%, and due to acetylcholine, 31%. Thus, papaverine was found to have a greater depressant action on the contraction evoked by transmural stimulation than on the contraction caused by the addition of exogenous agonists. The effect of papaverine, 10^{-6}-10^{-7}M, was not due to a reduction in the sensitivity of the effector cells, because papaverine in these concentrations did not reduce the res-

ponse of these preparations to exogenouse agonists. It indicates that the latter effect may be attributed to prejunctional effects of papaverine. However, further investigation is required to elucidate the precise actions of papaverine on the contraction induced by transmural-stimulation.

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