PHARMACOLOGICAL ANALYSIS OF ACETYLCHOLINE-INDUCED CONTRACTION IN MOUSE VAS DEFERENS

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Since preparations of the hypogastric nerve-vas deferens of the guinea-pig were first used in pharmacological experiments by Hukovic (1) and in electrophysiological experiments by Burnstock and Holman (2), these preparations have been used widely to study the pharmacology, physiology and histochemistry of the autonomic nervous system (3-8). Electrophysiological (9) and electronmicroscopic (10) studies in mouse vas deferens have also been performed, and subsequently the pharmacological properties, namely the effects of cholinergic and adrenergic drugs on the contraction induced by transmural stimulation and the autonomic innervation, were clarified (11).

During studies of the effect of acetylcholine (ACh) on the vas deferens of the mouse, we found that the contraction of the vas deferens induced by ACh showed a dual phase response which was different from that of the guinea-pig vas deferens. In the present paper, a study was made of the dual contraction induced by ACh in mouse vas deferens and evidence was found for the existence of nicotinic sites in the sympathetic nerve endings.

After the vas deferens was dissected from the mouse (ddY strain, 25-35 g), the serous coat was stripped off and the preparation was suspended in a 10 ml bath containing Tyrode solution at 32°C and aerated with 95% O₂ : 5% CO₂. The contraction of the vas deferens was recorded on a recticorder (Sanei 88) through a FD pick up (Nihon Kohden SB-IT-H).

In the mouse vas deferens, as shown in Fig. 1, the ACh-induced contraction was composed of two components of which one has a rapid "fast phase" (FP) and the other a lasting "slow phase" (SP). This biphasic contraction was dose-dependent. The SP was superior to the FP in concentrations of less than 3 \(10^{-5}\) g/ml of ACh but the FP
showed a much more rapid rate of increase at higher concentrations. In the experiments described below, the concentration of ACh used was 10^{-4} g/ml. The FP was only very slightly inhibited by atropine (3 \times 10^{-9} - 10^{-8} g/ml), but was completely abolished by hexamethonium (C_6, 10^{-5} g/ml) (Fig. 2b, c). This finding suggests that the FP is due to the stimulation of a nicotinic site by ACh and not due to the excitation of the muscarinic site known well in the vas deferens of guinea-pig and rat. Though the FP was blocked by a local anaesthetic, procaine (5 \times 10^{-5} g/ml), it was not blocked by tetrodotoxin even at high concentration (10^{-7} g/ml) which block completely the contraction of the vas deferens induced by transmural stimulation (Fig. 2d, e). Furthermore, the FP was abolished by a catecholamine (CA) depletor of the sympathetic nerve endings, 6-hydroxydopamine (6-OHDA, 100 mg/kg i.v., 24 hr prior to the experiment), and only the SP was left. As shown in Fig. 2f, g, the adrenergic blocking agents guanethidine (5 \times 10^{-7} g/ml) and phentolamine (10^{-8} g/ml) also showed a selective inhibition of FP.

These results indicate that the FP produced by a high concentration of ACh (10^{-4} g/ml) was due to the action of CA released by the excitation of the nicotinic site of which the existence was assumed in the sympathetic nerve endings. Since the FP was not abolished by tetrodotoxin, however, the action of ACh in this preparation might be tetrodotoxin resistant such as the descriptions of Su and Bevan (12) in the rat pulmonary artery, Katz and Miledi (13) in the squid stellate ganglion, Löffelhitz (14) in the rabbit heart and Goldenberg (15) in the rat ileum.

These facts indicate that the action of ACh is on the ganglion, though in the guinea pig and the rat, ganglion cells were found to be situated in the vicinity of the effector but not in the muscle layer (16–18). Such being the case, the existence of ganglion in the preparation isolated by the procedure used in these experiments was examined by lightmicroscopy. The preparation was fixed in 10\% formalin and cross-sections 10 \mu thick were cut. These sections were stained with haematoxylin and eosin. No ganglion cell was observed in any section. Therefore, the action of ACh on the ganglion could be excluded.

These experimental data might support the hypothesis of Burn and Rand (19) that the release of noradrenaline from adrenergic nerves was mediated by ACh.
The SP induced by ACh was not suppressed by Ca, procaine, tetrodotoxin, 6-OHDA, guanethidine and phentolamine, rather these agents demonstrated a tendency to potentiate. This effect was abolished by atropine. It is thus considered that the SP is due to the excitation of the muscarinic site on the muscle as is generally reported.

These facts suggest the existence of a tetrodotoxin resistant nicotinic site in sympathetic nerve endings in addition to that of the well-known muscarinic site as the active site of exogenous ACh in mouse vas deferens.

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