PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 200-201 (1978)

Dual Excitatory Effect of Acetylcholine in the Mouse Vas Deferens[1] (40020)

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Sympathomimetic effects of acetylcholine and other nicotinic agonists have been described in a variety of tissues (1). For instance, Daly and Scott (2) showed that close arterial injection of acetylcholine into the dog spleen caused contraction which was greatly reduced or abolished by hexamethonium, and by phenoxybenzamine, sympathetic denervation, or pretreatment with reserpine. Similar observations were made in cat and dog heart preparations by Lee and Shideman (3). Subsequent biochemical studies in cat and rabbit hearts provided direct evidence for release of norepinephrine by acetylcholine and other nicotinic agonists (4, 5). The absence of ganglion cells in many of the tissues studied (2, 3) suggested that the nicotinic receptors mediating the release of norepinephrine were located at adrenergic nerve terminals, a view supported by the failure of tetrodotoxin to prevent acetylcholine-induced release from cat spleen (6) or nicotine-induced release from guinea pig hearts (7). In the present study we have examined whether release of norepinephrine may contribute to the contractile response of the mouse vas deferens to acetylcholine. The experiments were prompted by a preliminary observation that the concentration-response curve of acetylcholine in this tissue was biphasic.

Materials and methods. Mice, weighing 30–40 g, were killed by a blow to the head. Vasa deferentia were removed and suspended in organ baths containing 25 ml of modified Krebs' solution of the following composition (millimolar): NaCl, 113; KCl, 4.8; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 25; and dextrose, 5.5. The solution was maintained at 37°C and continuously bubbled with 95% O_2:5% CO_2. Contractions of the smooth muscle were recorded isometrically using a Grass force-displacement transducer (FT.03) and model 5D polygraph. An initial tension of 0.5 g was applied and the tissues were equilibrated for 1 hr before drugs were applied to the bath. Concentration-response curves were obtained by adding increasing doses of acetylcholine at 15-min intervals. Acetylcholine was removed by washing when the contractile response had reached its peak. When the effects of hexamethonium, atropine, tetrodotoxin, and phentolamine on the responses to acetylcholine were examined, these drugs were applied for 1 hr prior to, and throughout, the period of exposure to acetylcholine.

Results and discussion. Concentration-response curves of acetylcholine are shown in Fig. 1. In untreated tissues, the mean concentration-response curve of acetylcholine was biphasic. Treatment with either a muscarinic antagonist (atropine) or a nicotinic antagonist (hexamethonium) separated the atypical curve into two simple concentration-response curves. The muscarinic effects of acetylcholine occurred in the range of concentrations from 10^{-6} to 10^{-4} *M*, while nicotinic effects were restricted to higher concentrations, approximately 10^{-4} to 3×10^{-3} *M*. The muscarinic receptors are presumably located on the smooth muscle cells. The nicotinic effects were mediated by release of norepinephrine since they were antagonized by phentolamine, suggesting that the nicotinic receptors are located on adrenergic neurons. The observation that tetrodotoxin did not inhibit the nicotinic effects suggests that action potentials are not involved in the release of norepinephrine by acetylcholine in this tissue. In other experiments responses of the mouse vas deferens to transmural electrical stimulation were inhibited by tetrodotoxin. Thus the results are consistent with the location of excitatory nicotinic receptors on adrenergic nerve terminals. Similar conclusions were reached from studies in cat and

[1] This work supported by National Institute of Neurological Diseases and Stroke Grant NS 080300.

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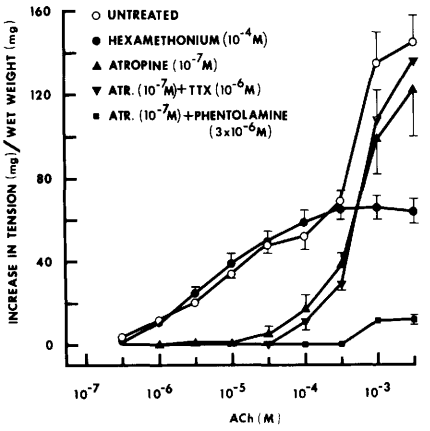

FIG. 1. Mean concentration–response curves of acetylcholine in the mouse vas deferens. Vertical bars represent standard errors. The number of observations at each point ranged from 4 to 12.

guinea pig hearts (5, 7), cat spleen (6), and rat seminal vesicle (8).

The control concentration–response curve in Fig. 1 differs from that obtained in the mouse vas deferens by Jones and Spriggs (9), who found that responses to acetylcholine were linear in the range of concentrations from 10^{-6} to 10^{-3} *M*. Furthermore, the mean maximum tension developed (0.48 g) was considerably less than we observed in controls (1.28 g), but similar to that in tissues treated with hexamethonium (0.66 g). It is possible that the relatively short time intervals between doses of acetylcholine (3 min) used by Jones and Spriggs resulted in attenuation of nicotinic responses due to autoinhibition as shown in the rabbit heart by Löffelholz (10).

Summary. Relatively low concentrations of acetylcholine ($< 10^{-4}$ *M*) contract the isolated mouse vas deferens by interaction with muscarinic receptors. Contractile responses to higher concentrations ($> 10^{-4}$ *M*) may be due to release of norepinephrine following interaction of acetylcholine with nicotinic receptors on adrenergic nerve terminals.

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Received September 27, 1977. P.S.E.B.M. 1978, Vol. 157.