

## Letter to the Editor

### NATURE OF CHOLINERGIC RECEPTORS ON THE ISOLATED FROG'S RECTUS ABDOMINIS MUSCLE

Sir,

Cholinergic receptors are classified into 2 major groups—muscarinic and nicotinic. These receptors have fairly specific and potent blocking agents *e.g.* atropine for muscarinic receptors, tubocurarine for nicotinic receptors on neuromuscular junction and pentolinium for nicotinic receptors on autonomic ganglionic synapse.

Isolated rectus abdominis muscle of frog is commonly used for bioassay of acetylcholine (ACh) and tubocurarine (2). It contains slow and fast contracting muscle fibres; the former respond to cholinergic drugs in the isolated preparation (6). We wish to emphasize that cholinergic receptors of the slow contracting fibres in isolated frog's rectus muscle behave paradoxically in some ways and that they are functionally less differentiated than those in the mammalian tissues.

Frogs' rectus abdominis muscle was mounted in 8 ml. capacity isolated organ bath in continuously oxygenated amphibian Ringer solution at room temperature (22-26° C). The agonists (and their effective doses/ml of bath fluid) were—acetylcholine chloride (0.06  $\mu$ g), carbachol chloride (0.3-0.6  $\mu$ g), suxethonium bromide (Brevidil, 0.3-0.6  $\mu$ g), arecoline chloride (0.4 mg) and potassium chloride (1 mg). The antagonists (and their approximately 50% inhibiting doses/ml of bath fluid) were—tubocurarine chloride (Tubarine, 0.3  $\mu$ g), atropine sulphate (5  $\mu$ g), oxyphenonium (Antrenyl, 3  $\mu$ g), adephenine (Trasentine, 4  $\mu$ g), pentolinium tartrate (Ansolysin, 12.5  $\mu$ g) and pilocarpine nitrate (0.3 mg). The antagonists were allowed to act for 3 minutes before the agonists were added. The agonists were allowed to act for 2 minutes after which 5 minutes were allowed for the tissue to recover. Contractures were recorded on smoked paper by a lever (X 10). Thirty eight tissues were used in this work.

Carbachol has greater nicotinic action and is less susceptible to cholinesterase than ACh (5). However, in this preparation, ACh was found to be about 10 times more potent than carbachol which is significant since their molecular weight are nearly equal (181 and 182 respectively).

In mammals suxethonium (molecular wt 472) has no significant muscarinic action but causes voluntary muscle paralysis by persistent depolarization of the neuromuscular junction. However, on the isolated frog rectus muscle it acted like and was about 1/10th as potent as ACh. The amphibian (and avian) voluntary muscles are stimulated by suxethonium and related drugs (3).

Arecoline is known to have mainly muscarinic action. In the isolated frog rectus muscle, on weight basis, ACh was about 6700 times more potent than arecoline.

Pilocarpine, in doses upto 20,000 times those of acetylcholine, was found devoid of any stimulant action. On the other hand, paradoxically it reduced contracture of the rectus muscle induced by ACh, carbachol and suxethonium. This confirms Coppee's finding on pilocarpine-induced blockade of ACh (4).

In the isolated frog's rectus muscle, stimulant action of ACh, carbachol and suxethonium was effectively blocked not only by tubocurarine but also by atropine (1) and its synthetic substitutes, pentolinium and pilocarpine. The antagonism was short-lived and the tissues recovered their sensitivity to agonists usually within 5-19 minutes. On increasing the dose of acetylcholine, carbachol or suxethonium, inhibitory action of the antagonists could be overcome. These findings suggest reversible, competitive nature of the action of the antagonists. Inhibitory potency of the individual antagonists against the 3 cholinergic agonists was nearly identical. On weight basis, tubocurarine was about 1,040 and 1,000 times more potent than atropine, pentolinium and pilocarpine respectively.

Potassium chloride produced contracture which is due to direct membrane depolarization. Therefore, its action could not be blocked by tubocurarine, atropine, pentolinium, or pilocarpine. It was used in this work only as a control.

Thus, the response of isolated frog's rectus muscle to cholinergic drugs is not related to their muscarinic or nicotinic potency. Pilocarpine as well as cholinergic antagonists of diverse sites and mechanisms block the stimulant action of cholinergic drugs. Therefore, unlike most of cholinergic receptors in the mammals, those on the slow contracting fibres of frog's rectus abdominis muscle are functionally less differentiated.

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