Biochemical Pharmacology, Vol. 30, No. 19, pp. 2713–2714, 1981.
Printed in Great Britain.

0006-2952/81/192713-02 $02.00/0
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SHORT COMMUNICATIONS

Effect of Tityus toxin and sensory stimulation on muscarinic cholinergic receptors *in vivo*

(*Received* 8 *January* 1981; *accepted* 9 *April* 1981)

Muscarinic receptors in the rat brain have been charactered in detail using tritiated agonists and antagonists. The extent of the binding of the antagonist[^{3}H]-*N*-methyl atropine to muscarinic receptors of synaptosomal membranes has been shown to diminish significantly following exposure to either electrical pulses or a depolarizing chemical such as veratrine [1–4]. The same kind of stimuli have been found to enhance the release of acetylcholine [5] and other neurotransmitter amino acids [6, 7] from incubated synaptosomal preparations in a fashion which suggests that they mimic the performance of nerve terminals *in vivo*. A most potent depolarizing agent of this type is Tityus toxin, which has been isolated from the venom of the Brazilian yellow scorpion [8]. Its actions and those of other depolarizing drugs, such as veratrine, are reversible and inhibited by low concentrations of tetrodotoxin [9–11]. The question therefore arises as to whether the *in vitro* effects on receptor populations produced by depolarising treatment, also occur *in vivo* as a result of neural activity and synaptic activation. This paper reports evidence that receptor population changes of this kind *do* occur *in vivo*.

The experiments were performed on female Sprague–Dawley rats (200–250 *g*) kept under light anaesthesia (as tested by a positive corneal reflex) by using an avertine–atropine solution (250 and 1.7 mg/kg). In the experiments the skull was exposed over the sensori-motor cortex and two small plastic cups (2 mm dia) were fixed with dental cement (experimental details as described previously [12]). Tityus toxin (50 nM) solution was added to the cup over the right sensori-motor cortex (stimulated side), whilst the left cup was filled with sterile normal saline (0.85 per cent w/v). Animals were treated with Tityus toxin for 30 min. Tetrodotoxin (1 μM) was added when appropriate. Unilateral electrical stimulation of the brachial plexus was performed via electrodes implanted around the plexus [12] and employed pulses with the following specification: 3–6 V amplitude; 1–3 mA current; 1 m sec pulse duration and 2–3 Hz frequency. Brachial plexus stimulation was applied for 30 minutes and always evoked clearly visible muscular jerking in the ipsilateral forelimb; clonic seizures were never seen to occur.

At 30 min after treatment with electrical stimulation or Tityus toxin, the animals were sacrificed and synaptosomes were prepared from the sensori-motor cortex as described by Gray and Whittaker [13] with the modification of Bradford *et al.* [14]. The synaptosomes were lysed in 10 mM phosphate buffer, pH 7.0 at 0°, and the pellet of synaptosomal membranes obtained after centrifugation (100,000 *g*, 10 min) was stored at −80°. Subsequent receptor binding assays (muscarinic acetylcholine receptors) were performed [1] using saturating concentrations of ^{3}[H]-*N*-methyl scopolamine (NMS), (10 Ci/mmole, 10^{-8} M), and a rapid centrifugation method [3]. Non-specific binding, determined by incubating membranes and [^{3}H]-NMS with a high concentration (1 μM) of 3 quinuclidinylbenzilate, was in general less than 10 per cent of the specific binding.

Synaptic membranes prepared from control animals showed a specific binding of [^{3}H]-*N*-methyl scopolamine of 2.65 ± 0.08 (n = 16) nmoles/g protein. In these control animals the electrodes were fixed around the brachial plexus but no stimulation was applied. In addition, the two cups were in position over the sensorimotor cortex, but these were filled with normal sterile saline.

Addition of Tityus toxin (50 nM) to one cup resulted in a 16 per cent decrease in [^{3}H]-NMS binding which was significantly different ($P < 0.05$) from levels in the control untreated animals as well as the levels in the untreated contralateral side of the same animal (Table 1). This decrease in binding was completely blocked by tetrodotoxin. Unilateral stimulation of the brachial plexus caused a similar 18 per cent decrease in binding but in this case the decrease in receptor levels was observed on *both* sides of the cortex (Table 1).

The effects of stimulation of one cortex, either chemically or by sensory stimulation, could be expected to spread to the contralateral homotopic area via the corpus callosum. Thus, detection of a change by comparison with the contralateral area would be more difficult than by comparison with equivalent cortex of untreated, sham-operated animals. This is clearly the explanation of the difficulty in detecting an effect due to brachial plexus stimulation in the data of Table 1, where it can be seen that *both* hemispheres of treated animals show a diminution in receptor population. This bilateral response to unilateral stimulation contrasts with the pattern of transmitter release. Our own previous work shows that detectable transmitter release is confined to the contralateral cortex [9, 11]. However, a change in receptor populations in the excised tissue may be more easily detected than transmitter release signals which are reduced in size by reuptake processes.

This *in vivo* loss of cholinergic muscarinic receptor is likely to be the result of the evoked neural activity rather than due to any direct action of the stimuli themselves. Thus, the change was seen to follow *both* direct chemical stimulation and indirect stimulation through sensory pathways. *In vitro* studies with incubated synaptosomes have shown that a similar loss evoked by electrical pulses and veratrine is inhibited by tetrodotoxin (1 μM) indicating that the loss is coupled to the depolarization event itself [4]. The action of Tityus toxin was also prevented in the present *in vivo* experiments. It is known that both, electrical stimulation and Tityus toxin treatment, cause a specific release of transmitter amino acids [9, 10] and of acetylcholine *in vivo*. These effects too are tetrodotoxin-sensitive and are reversible [11].

Thus, the present report employing an *in vivo* preparation provides additional evidence that neural activity and synaptic activation can lead to a modulation of the muscarinic cholinergic receptor population located in synapses located in the CNS.

Acknowledgements—This project was supported by an MRC Programme Grant. M. M. Boyar was supported by an MRC postgraduate studentship. A. S. Abdul-Ghani would like to thank IBRO/UNESCO for a travel grant. J. Coutinho-Netto was supported by a grant from the State São-Paulo Foundation (FAPESP), Brazil.

Table 1. Effect of brachial plexus stimulation and tityus toxin on muscarinic cholinergic receptor populations of synaptic membranes: comparison between hemispheres

Treatment	Unstimulated cortex (U.S.)	Stimulated cortex (S)	Ratio S/U.S. per cent
Sham-operated animals (control)	2.65 ± 0.08 (16)		
Tityus toxin	2.70 ± 0.16 (6)	2.22 ± 0.14 (6)§	83 ± 4 (6)†
Tityus toxin and tetrodotoxin	2.70 ± 0.09 (4)	2.67 ± 0.07 (4)	99 ± 3 (4)*
Brachial Plexus stimulation	2.22 ± 0.10 (10)	2.13 ± 0.12 (10)‡	96 ± 3 (10)*

Values, each from a separate animal, are expressed in nmoles/g protein and are mean ± S.E.M. for the number of experiments indicated in brackets.

Comparison is with contralateral cortex from the same animals where treatment was applied.

* Not significantly different from 100.

† Significantly different from 100 $P < 0.005$.

‡ A mean significant decrease of 18 per cent from control (2.65 ± 0.08) $P < 0.005$.

§ A significant decrease of 16 per cent from control (2.65 ± 0.08) $P < 0.05$.

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