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ASYNCHRONOUS POSTGANGLIONIC FIRING FROM THE CAT SUPERIOR CERVICAL SYMPATHETIC GANGLION TREATED WITH NEOSTIGMINE

BY

C. TAKESHIGE AND R. L. VOLLE

From the Department of Pharmacology, Schools of Medicine, University of Pennsylvania, Philadelphia, U.S.A.

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Neostigmine injected intra-arterially to the superior cervical ganglion of the cat evoked an asynchronous postganglionic nervous discharge in both normal and denervated superior cervical ganglia. This asynchronous firing was enhanced by tubocurarine but blocked by small doses of atropine. In addition, the responses evoked by acetylcholine in ganglia treated with neostigmine were characterized by two components. The first was blocked by tubocurarine and the second by atropine. Asynchronous firing evoked by repetitive preganglionic nerve stimulation of ganglia treated with neostigmine was blocked by atropine but not tubocurarine. It is suggested that neostigmine (1) has actions on ganglia other than those attributable to inactivation of cholinesterase, (2) may possess both pre- and post-synaptic sites of action, and (3) may unmask a cholinoceptive site which can be blocked by atropine.

The asynchronous postganglionic nerve discharge from the superior cervical ganglion of the cat after intra-arterial administration of dyflos (Volle & Koelle, 1961 ; Volle, 1962a) or after eserine (Takeshige & Volle, 1962) is consistent with the suggestion of Feldberg (1945) that acetylcholine is liberated spontaneously from the nerve terminals of resting ganglia. However, unlike the postganglionic responses to stimulation of the preganglionic nerve, the discharge induced by the anticholinesterase agents was enhanced by tubocurarine and blocked by small doses of atropine (Volle, 1962a ; Takeshige & Volle, 1962). Furthermore, the responses to acetylcholine of the ganglia treated with eserine or by conditioning with repetitive preganglionic nerve stimulation were characterized by a bimodal pattern. The first component of the response was blocked by tubocurarine but not by atropine whereas the second was resistant to block by tubocurarine but was antagonized by small doses of atropine (Takeshige & Volle, 1962). Consequently, it was suggested that the mechanism underlying the discharge differed from the usual form of ganglionic transmission and, further, that these anticholinesterase agents produced changes in the ganglia which were distinct from those which were caused by the inhibition of the cholinesterases.

In view of the direct excitation by neostigmine at the skeletal neuromuscular junction (Riker & Wescoe, 1946) and at superior cervical ganglion of the cat (Mason, 1962a and b), the present experiments on the effects of neostigmine on normal (" decentralized ") and on chronically denervated ganglia of the cat were undertaken.

METHODS

All experiments were performed with the superior cervical ganglia of cats anaesthetized either with urethane (1 g/kg of body weight, intraperitoneally) or with a mixture of sodium diallylbarbitone with urethane (Dial, 0.7 ml./kg, intraperitoneally). The results with both anaesthetic procedures were similar.

Preparation of the normal ganglion

Following intubation of the trachea, a deep cervical well was prepared by removing the oesophagus and the larynx with a stump of the trachea. The left superior cervical ganglion was exposed, the cervical sympathetic trunk dissected free from the vagus nerve and the common carotid artery, and the external carotid branch of the postganglionic nerve separated from the external carotid artery. Skin flaps were tied to a metal frame and the resulting well was filled with medicinal liquid paraffin. The pre- and post-ganglionic nerves were cut and suspended on glass hooks in the paraffin.

Platinum electrodes were used for stimulation of the preganglionic nerve and for recording postganglionic nerve action potentials. An electronic stimulator provided rectangular pulses, the parameters of which were variable. The stimulus duration was usually 0.1 msec. Stimuli were isolated from earth. The action potentials evoked were amplified by capacitance-coupled amplifiers and observed on a dual beam oscilloscope.

The asynchronous postganglionic discharges evoked by chemical agents were amplified so that a 20 μV signal produced a vertical deflexion of 1 cm on the oscilloscope, and were recorded photographically. The frequency response of the amplifier was adjusted so that, at 35 and at 2,000 cycles/sec, the gain was reduced to half.

All branches of the common carotid artery including the external carotid artery but excepting those supplying the ganglion directly were tied and a 27-gauge needle, fitted to a holder and clamped to the framework, was inserted into the common carotid artery for the intra-arterial injection of drugs. The volume of injection never exceeded 0.2 ml. All of the drugs were dissolved in 0.9% saline. Clotting in the needle was prevented by the prior administration of heparin (300 units/kg, intravenously).

Chronically denervated preparations

Chronic denervation of the ganglia was performed with cats anaesthetized with pentobarbitone (30 mg/kg, intraperitoneally) by resecting a 1 cm segment of the cervical sympathetic trunk 5 to 7 cm caudal to the ganglion. The central end of the nerve was tied, the wound closed, and the animals treated prophylactically with penicillin and dihydrostreptomycin. The ganglia were prepared, 10 to 20 days later, for recording in the manner described above for normal ganglia. The absence of a postganglionic response to stimulation of the preganglionic nerve on the same side was taken as evidence of complete denervation of the ganglion cells.

The drugs used were: neostigmine bromide, atropine sulphate, (+)-tubocurarine chloride, hexamethonium chloride and acetylcholine chloride. All of the doses are expressed in terms of the salts, and refer to intra-arterial injections.

RESULTS

Asynchronous postganglionic nerve discharge evoked by neostigmine in normal and denervated ganglia

In normal ganglia, neostigmine (20 to 100 μg) evoked regularly a low amplitude postganglionic asynchronous discharge arising in 10 to 20 sec and persisting for 4 to 15 min (Fig. 1). Stimulation of the preganglionic nerve with trains of supramaximal shocks at rates of 20 to 30 shocks/sec for 10 to 30 sec following the

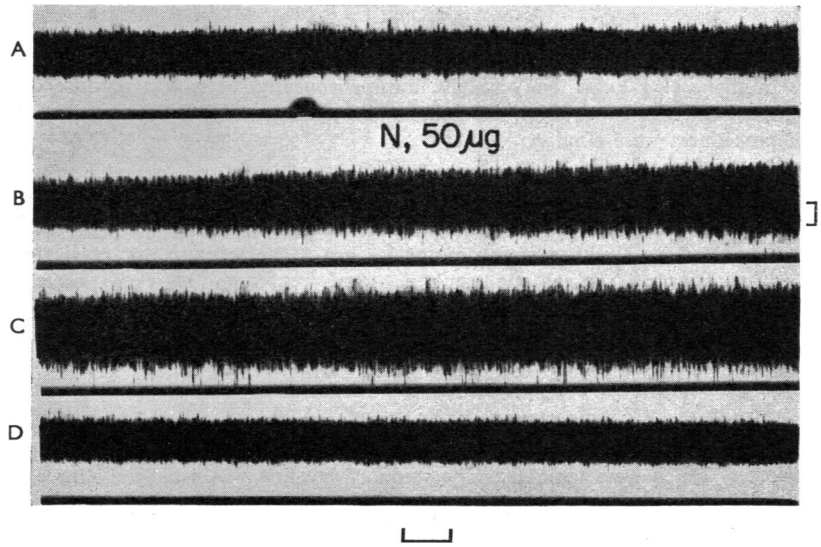

Fig. 1. Asynchronous postganglionic discharge evoked by neostigmine (50 μg, intra-arterially) of superior cervical ganglion of the cat. Records A, B, and C are continuous. Record D was obtained 20 min after C. Vertical calibration is 10 μV; horizontal calibration is 1 sec. Deflexion of base-line signals injection of neostigmine (N).

dissipation of the asychronous discharge resulted in a re-emergence of the asynchronous postganglionic firing. In addition, the prior conditioning of the ganglia with repetitive supramaximal preganglionic nerve stimuli at rates of 20 to 30 shocks/sec for 10 to 30 sec reduced the amount of neostigmine by approximately ten-fold required to elicit the asynchronous discharge.

Somewhat higher doses of neostigmine were needed to evoke a postganglionic asynchronous discharge in denervated ganglia. In each of five denervated ganglia, postganglionic firing followed the intra-arterial administration of 400 μg of neostigmine, while lower doses of neostigmine (100 to 200 μg) evoked a postganglionic response in only one experiment. The character of the response to neostigmine in denervated ganglia differed from that in normal ganglia in that it was immediate in onset and persisted for only 30 to 60 sec.

Differential block by tubocurarine and atropine of the responses of the ganglia treated with neostigmine

The asynchronous firing produced by neostigmine in normal ganglia was enhanced by tubocurarine (150 to 900 μg ; Fig. 2, C). Firing was also evoked by tubocurarine following the dissipation of the firing due to neostigmine. Similar doses of tubocurarine reduced by 80 to 100% the amplitude of the postganglionic action potentials evoked by repetitive supramaximal stimulation of the preganglionic nerve at a rate of 0.5 shocks/sec (Fig. 2, C II). On the other hand, atropine, in doses (0.5 to 1.0 μg) which had no discernible effect on the responses of the ganglia to preganglionic stimulation, blocked completely the asynchronous discharge produced

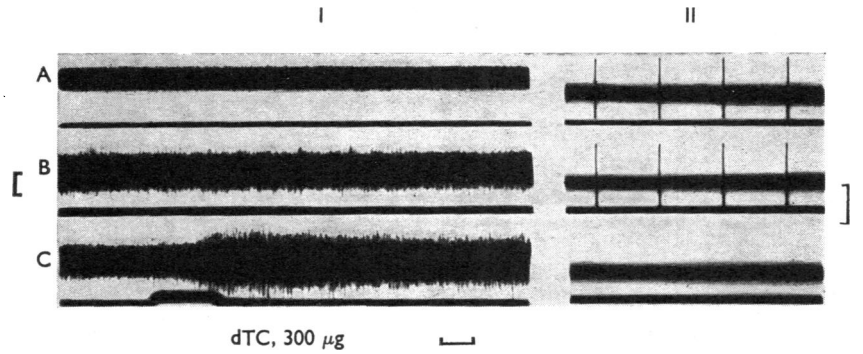

Fig. 2. Effects of tubocurarine (300 μg, intra-arterially) on, I, postganglionic responses evoked by neostigmine (100 μg, intra-arterially) and on, II, repetitive preganglionic stimulation at a rate of 0.5 shocks/sec. Records: A, I and II, controls; B, responses evoked by neostigmine (100 μg, intra-arterially; B, I) and preganglionic nerve stimulation (B, II) recorded approximately 1 min after the administration of neostigmine; C, I and II, effects of tubocurarine (dTC; 300 μg, intra-arterially) in augmenting discharge evoked by neostigmine (C, I) and in blocking postganglionic action potentials (C, II, recorded 5 min after the administration of neostigmine). Vertical calibrations on right and left are 1 mV and 10 μV, respectively. Horizontal calibration is 1 sec.

by neostigmine (Fig. 3, B). Similarly, atropine administered prior to the injection of neostigmine prevented the occurrence of the discharge. The block of asynchronous firing by atropine persisted for several hours and could be antagonized either by repeated injections of neostigmine (50 to 100 μg) or by repetitive stimulation of the preganglionic nerve (20 to 30 shocks/sec for 10 to 20 sec). The discharge evoked by neostigmine in denervated ganglia could also be blocked by atropine but not by tubocurarine.

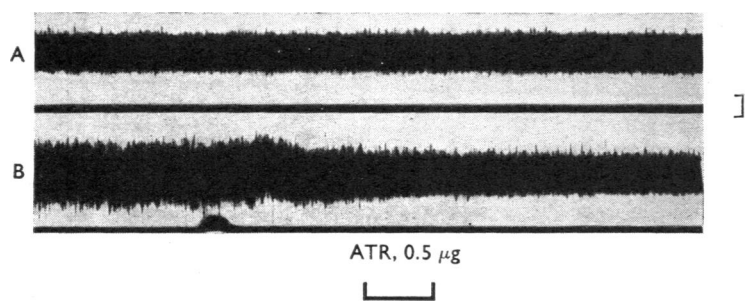

Fig. 3. Block by atropine (ATR; 0.5 μg, intra-arterially, at signal in B) of the asynchronous postganglionic discharge evoked by neostigmine (50 μg, intra-arterially). A, control; B, response approximately 1 min after the injection of neostigmine. Vertical and horizontal calibrations are 10 μV and 1 sec, respectively.

The postganglionic response to injected acetylcholine (2 to 10 μg) of ganglia treated with neostigmine was bimodal (Fig. 4). Like the response of eserine-treated ganglia to acetylcholine (Takeshige & Volle, 1962), the first component of the response was blocked by tubocurarine, and the second by small doses of atropine (Fig. 4, B).

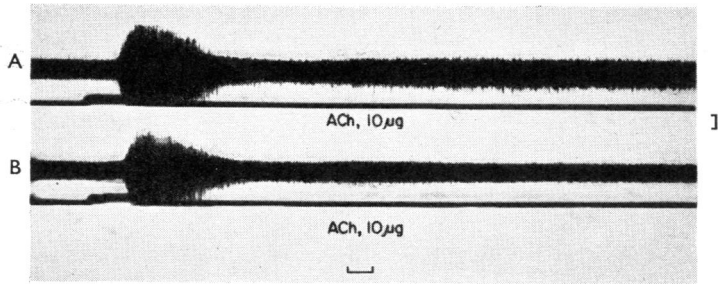

Fig. 4. Postganglionic response to acetylcholine (ACh; 10 μg, intra-arterially, at signal) of superior cervical ganglion treated with neostigmine (50 μg, intra-arterially). A, bimodal response to acetylcholine before atropine (1 μg, intra-arterially); B, response to acetylcholine 5 min after the injection of atropine. Vertical and horizontal calibrations are 10 μV and 1 sec, respectively.

A similar differential action of the two drugs was observed on the asynchronous firing evoked by repetitive preganglionic nerve stimulation with ganglia treated with neostigmine. Tubocurarine, administered in doses (600 to 1,500 μg) which blocked completely the postganglionic response evoked by stimulation of the preganglionic nerve with supramaximal stimuli at 0.5 shocks/sec, did not block the asynchronous firing which occurred during and after the preganglionic volleys (Fig. 5, B), while atropine (1 μg) abolished the discharge induced by the repetitive volleys (Fig. 5, C).

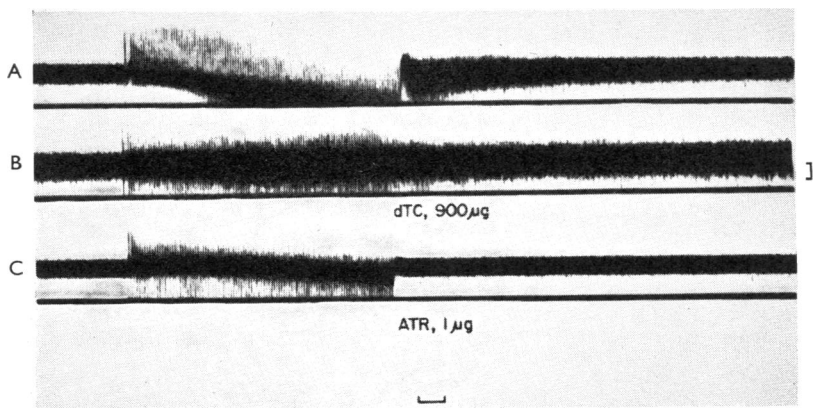

Fig. 5. Effects of tubocurarine (dTC; 900 μg, intra-arterially) and atropine (ATR; 1 μg, intra-arterially) on postganglionic responses to repetitive preganglionic nerve stimulation (10 shocks/sec for 10 sec) of superior cervical ganglion treated with neostigmine (150 μg, intra-arterially). A, control response evoked by preganglionic stimulation following administration of neostigmine; B, response evoked by preganglionic stimulation after the injection of tubocurarine (note the increase in background activity which was evoked by tubocurarine prior to onset of preganglionic stimulation); C, block by atropine of asynchronous firing of ganglion cells evoked by preganglionic stimulation. Vertical and horizontal calibrations are 10 μV and 1 sec, respectively.

DISCUSSION

That neostigmine evoked an asynchronous postganglionic discharge which was enhanced by tubocurarine and blocked by atropine indicates that this anticholinesterase agent had actions on the ganglia which were similar to those produced by dyflos (Volle, 1962a ; Komalahiranya & Volle, 1962) and by eserine (Takeshige & Volle, 1962). On the other hand, neostigmine differed from dyflos and from eserine in that it also evoked postganglionic firing in denervated ganglia. The latter finding is compatible with the suggestion of Mason (1962a, b) that neostigmine directly excites the ganglion cells. However, the prolonged block by small doses of atropine of the responses evoked by neostigmine also suggests that these actions of neostigmine were due to the activation of receptor sites which were pharmacologically different from those usually associated with transmission in sympathetic ganglia. This latter proposal is consistent with earlier findings that the responses of sympathetic ganglia to pilocarpine (Trendelenburg, 1954) and the pressor response evoked in dogs by neostigmine (Long & Eckstein, 1961 ; Hilton, 1961 ; Levy & Ahlquist, 1962) could be blocked by atropine.

However, the excitatory actions of neostigmine described by Mason (1962a) were not affected by atropine. The most likely explanation for the discrepancy between his findings and ours is that the effects of neostigmine were studied on different cell groups within the ganglia. Mason (1962a) used the contraction of the nictitating membrane as an index of ganglionic activity, whereas we recorded action potentials from the small postganglionic nerve which passes from the superior cervical ganglion to the external carotid artery. There is good electrophysiological evidence for the existence of functionally distinct neural pathways through the cat superior cervical ganglion (Bishop & Heinbecker, 1932 ; Eccles, 1935). Furthermore, the several cell groups differ in their sensitivities to ganglionic blocking or stimulating drugs (Mainland & Shaw, 1952 ; Hertzler, 1961 ; Volle, 1962b). Nonetheless, there is good agreement between our findings and the conclusion of Mason (1962a) that neostigmine acts directly on sympathetic ganglia in addition to its more widely known anticholinesterase action.

Inactivation of the cholinesterases of the ganglia by the anticholinesterase agents does appear to play an important role in the genesis of the postganglionic firing because: (a), with the exception of neostigmine, asynchronous postganglionic firing did not occur in chronically denervated ganglia in which most of the acetylcholinesterase had disappeared (Koelle & Koelle, 1959) ; (b), the onset of the firing was delayed ; (c), the durations of firing produced by the several anticholinesterase agents were consistent with classification of the drugs as reversible or irreversible inhibitors ; and (d), the postganglionic firing was produced by anticholinesterase agents of diverse chemical structures. Accordingly, the suggestion (Takeshige & Volle, 1962) that the asynchronous firing produced by eserine was due to an anticholinesterase action which either preserved the transmitter liberated spontaneously or enhanced the release of the transmitter is also applicable to the firing produced by neostigmine.

Moreover, this proposal accords with the observation that the sensitivity to neostigmine of the cells giving rise to the external carotid nerve was reduced in

denervated ganglia and was increased in normal ganglia by repetitive stimulation of the preganglionic nerve. Similar changes in the responses to ganglionic stimulants of this cell group in denervated and conditioned ganglia have been described and attributed to a presynaptic component in the actions of the drugs on the ganglia (Volle & Koelle, 1961; Volle, 1962c). Thus, neostigmine may have acted both on the preganglionic nerve filaments and on the ganglion cells.

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