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A COMPARISON OF THE GANGLION POTENTIALS AND BLOCK PRODUCED BY ACETYLCHOLINE AND TETRAMETHYLAMMONIUM

BY

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Potentials recorded from the surface of the superior cervical ganglion of cats after an intra-arterial injection of acetylcholine were characterized by a complex waveform which depended on the amount of drug administered. Small doses of acetylcholine evoked a potential consisting of low amplitude hyperpolarization followed by low amplitude depolarization. Somewhat larger doses of acetylcholine caused a triphasic potential containing an initial period of depolarization in addition to the periods of hyperpolarization and delayed depolarization. Still larger doses of acetylcholine produced usually a monophasic wave of depolarization. Small doses of atropine prolonged the initial period of depolarization and prevented the hyperpolarization and delayed depolarization. Hexamethonium reduced or abolished the initial depolarization and enhanced or unmasked the hyperpolarization. The block of transmission occurring during the falling phase of the initial depolarization or during the hyperpolarization was antagonized by atropine. Unlike acetylcholine, tetramethylammonium produced only a prolonged ganglion depolarization which was unaffected by atropine and blocked by hexamethonium. The block of transmission by tetramethylammonium was partially prevented by atropine. These findings support the proposals that three pharmacologically distinctive cholinoceptive sites are present in sympathetic ganglia and, further, that activation of a cholinoceptive site sensitive to atropine may be involved in the block of transmission produced by acetylcholine and related drugs.

The depression of synaptic transmission in sympathetic ganglia by ganglion stimulating agents such as acetylcholine, tetramethylammonium and nicotine is attributed usually to depolarization of the ganglion cells (Paton & Perry, 1953 ; Lundberg & Thesleff, 1953 ; Mason, 1962). This concept of ganglion block is based on the observation that the inhibition of transmission produced by these agents coincides with the development of a negative ganglion potential (depolarization). On the other hand, some evidence has been presented which indicates that the block of transmission by acetylcholine is not related always to depolarization. Krivoy & Wills (1956) have reported that the transmission of impulses in isolated sympathetic ganglia exposed to acetycholine returned to normal during sustained ganglion depolarization. A similar separation of ganglion depolarization and block has been described in superior cervical ganglia treated with small doses of atropine (Takeshige, Pappano, DeGroat & Volle, 1963) ; in this study it was observed that,

although the block of transmission occurred during the period of depolarization produced by acetylcholine, atropine prevented the block of transmission by acetylcholine, but had no effect on the drug-induced negative potential.

In contrast to acetylcholine, acetyl-β-methylcholine (methacholine) produced a block of transmission which was associated with a positive ganglion potential (hyperpolarization). Atropine prevented both these actions of methacholine. On the basis of these observations, the suggestion was made that the block of synaptic transmission by methacholine was due to the activation of an inhibitory process which, in turn, was sensitive to block by atropine (Takeshige *et al.*, 1963). The presence of inhibitory processes in sympathetic ganglia has been considered by Marazzi (1939), Bülbring (1944), Laporte & Lorente de Nó (1950), Lundberg (1952), Dempsher, Tokumaru & Zabara (1959), Eccles & Libet (1961) and Tauc & Gerschenfeld (1962).

Although the relationships between the inhibition of transmission and the polarity of the ganglion potentials differed for acetylcholine and methacholine, the antagonism by atropine of their effects on transmission suggests that both agents may share a common mechanism of action. The experiments described below were designed to test further this possibility.

METHODS

Twenty-six cats were anaesthetized with a mixture of sodium diallylbarbitone and urethane (Dial, 0.7 ml./kg, intraperitoneally). After tracheal cannulation, 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 was dissected free from the vagus nerve and the common carotid artery, and the external carotid branch of the postganglionic nerve was 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 postganglionic nerves were cut and suspended on glass hooks in the paraffin.

All branches of the left common carotid artery except the external carotid artery and those supplying the ganglion directly were tied, and a 27-gauge needle, fitted to a holder and clamped to the framework, was tied into the common carotid artery for the intra-arterial injection of drugs. A small clamp was placed on the external carotid artery before administration of drugs and was removed between drug injections. 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 U/kg, intravenously).

Recording postganglionic nerve action potentials

Platinum electrodes were used for recording postganglionic nerve action potentials. One electrode was placed on the crushed end of the postganglionic nerve; the other, directly on the nerve proper. Action potentials were amplified by an RC amplifier and displayed on one beam of a dual-beam oscilloscope.

The asynchronous postganglionic discharges evoked by chemical agents were amplified so that a 20 μV signal produced a vertical deflection of 1 cm on the oscilloscope, and they were photographed on moving paper. The frequency-response of the amplifier was adjusted so that, at 35 and at 2,000 cycles/sec, the gain was reduced to half. The time scale was provided by the speed at which the paper moved through the recording camera.

Recording ganglion potentials

Ganglion potentials were recorded through silver-silver chloride bipolar electrodes. One electrode was placed in direct contact with the surface of the ganglion; the other, on the

crushed end of the postganglionic nerve. The position of the electrode on the ganglion was maintained throughout the experiment. The potentials evoked by the drugs were coupled directly by a cathode-follower circuit to a DC preamplifier. The ganglion potentials were viewed and recorded as described above for postganglionic nerve action potentials. The recording system was arranged so that an upward deflexion on the records indicated negativity. Throughout this paer ganglion negativity and positivity will be referred to as depolarization and hyperpolarization, respectively.

Stimulation of the preganglionic nerve

Platinum electrodes were used for stimulation of the cervical sympathetic trunk. An electronic stimulator provided rectangular pulses, the parameters of which were variable. The stimulus duration was 0.1 msec. Stimuli were isolated from earth. Supramaximal shocks, 2 V above that required to evoke the largest amplitude of the ganglion and postganglionic action potentials, were delivered at 0.5 shocks/sec in all experiments.

Drugs

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

RESULTS

Ganglion potentials and postganglionic nerve action potentials

The contour of the ganglion potential evoked by acetylcholine was determined by the amount of acetylcholine administered. Although the doses of acetylcholine required to evoke the several potentials varied for each ganglion, the following general dose/response relationships were observed in each experiment. The ganglion potential evoked by large doses of acetylcholine (50 to 200 μg) usually consisted of a single wave of depolarization (Fig. 1). In an occasional experiment, however, the negative potential was followed by a period of hyperpolarization which persisted for 30 to 90 sec. The postganglionic discharges evoked by these doses of acetylcholine often consisted of two periods of firing. The first burst of firing occurred during the rising phase of the ganglion negative potential. The second component of the firing was inconstant, and occurred 2 to 4 sec after the initial burst of firing, at a time when the initial depolarization had subsided. During the second period of firing, the ganglion potential was either slightly negative or at the resting level. When somewhat smaller doses (2.5 to 20 μg) of acetylcholine were injected, the ganglion potential evoked was triphasic (Fig. 1). Postganglionic firing was produced usually by 10 to 20 μg of acetylcholine and was present only during the rising phase of the initial period of depolarization. Smaller doses of acetylcholine (0.25 to 1 μg) evoked a ganglion potential consisting of hyperpolarization and delayed depolarization (Fig. 1). Although both potentials were of low amplitude, they were observed regularly in each preparation. In many of the experiments, acetylcholine (0.25 to 0.50 μg) produced only the delayed depolarization ; postganglionic discharges were not seen after the injection of these small doses of acetylcholine. Thus, with increasing doses of acetylcholine, the shape of the evoked ganglion potentials changed from delayed low amplitude depolarization to hyperpolarization followed by depolarization, to the triphasic complex of depolarization, hyperpolarization and depolarization and, finally, to a single wave of depolarization.

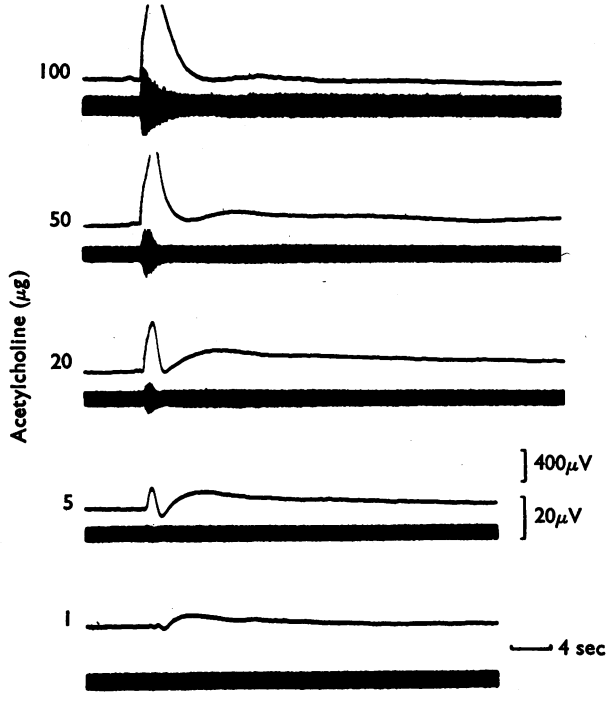

Fig. 1. Ganglion potentials (top) and postganglionic nerve action potentials (bottom) evoked by various amounts of acetylcholine (doses in μg on the left) and recorded simultaneously from the surface of the ganglion and a postganglionic nerve of a superior cervical ganglion of a cat. The vertical calibrations refer to the ganglion potentials (top) and the postganglionic responses (bottom) respectively.

Unlike the ganglion potentials evoked by acetylcholine, those occurring after the administration of tetramethylammonium (0.1 to 12 μg) consisted only of a prolonged depolarization (Fig. 2). The postganglionic discharge produced by tetramethylammonium (1 to 12 μg) was characterized by a single discharge and coincided primarily with the rising portion of the negative ganglion potential.

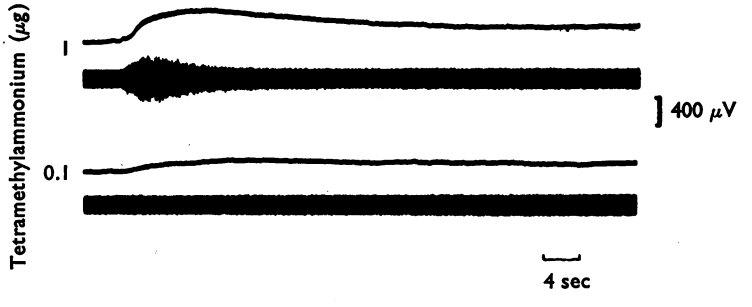

Fig. 2. Ganglion potentials (top) and postganglionic response (bottom) evoked by tetramethylammonium (doses in μg on the left) and recorded simultaneously. The vertical calibration refers to the top tracing of each pair of records.

Effects of hexamethonium and atropine on ganglion potentials evoked by acetylcholine and tetramethylammonium

Hexamethonium (300 μg to 1 mg) injected into the arterial supply of the ganglion had no measurable effect on nonstimulated ganglia. However, the contours of the ganglion potentials evoked by acetylcholine were altered considerably in ganglia treated previously with hexamethonium (Fig. 3). Whereas hexamethonium reduced or abolished the initial depolarization produced by moderate doses of acetylcholine (10 to 20 μg), it enhanced the hyperpolarization which followed. Similarly, the

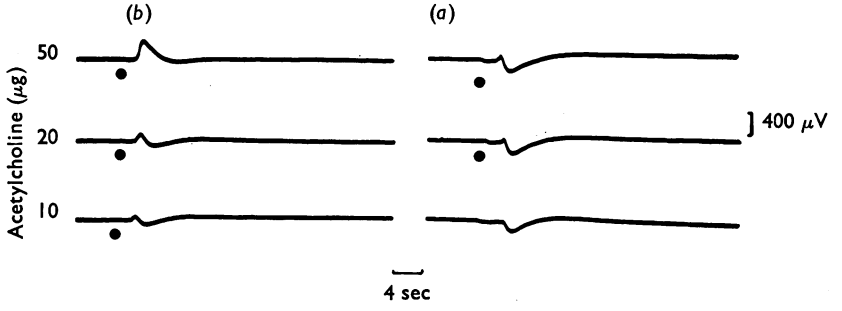

Fig. 3. The effects of hexamethonium on the ganglion potentials produced by 10, 20 and 50 μg of acetylcholine. The responses of the untreated ganglion to acetylcholine are shown in column *b* (left) and those recorded after the administration of hexamethonium are shown in column *a* (right). Since the effects of hexamethonium given intra-arterially were reversible, 1 mg of hexamethonium was injected 1 min before each injection of acetylcholine. The dots below each tracing signal the injections of acetylcholine.

depolarization produced by 50 μg of acetylcholine was converted by hexamethonium to a low amplitude depolarization followed by a pronounced hyperpolarization and by a delayed depolarization. Similar changes in the ganglionic responses to acetylcholine were produced by tubocurarine (150 to 500 μg). The ganglion potentials evoked by tetramethylammonium were reduced greatly or blocked completely by these doses of hexamethonium. It is noteworthy that tetramethylammonium, in contrast to acetylcholine, did not evoke hyperpolarization in ganglia treated previously with hexamethonium.

Although atropine (1 μg) had no effect either on the amplitude of the initial depolarization or on the postganglionic firing evoked by acetylcholine it prolonged the falling phase of depolarization and abolished completely the hyperpolarization produced by acetylcholine (Fig. 4). In addition, atropine unmasked the initial phase of depolarization after small doses of acetylcholine. Larger doses of atropine (2 μg) were required to block the late ganglion depolarization. Atropine (1 to 2 μg) had no detectable effect on the ganglion potentials produced by tetramethylammonium.

Relationship between ganglion potentials and block produced by acetylcholine and tetramethylammonium

As reported previously, the block of transmission produced by relatively large doses of acetylcholine (100 to 250 μg) occurred during the period of ganglion

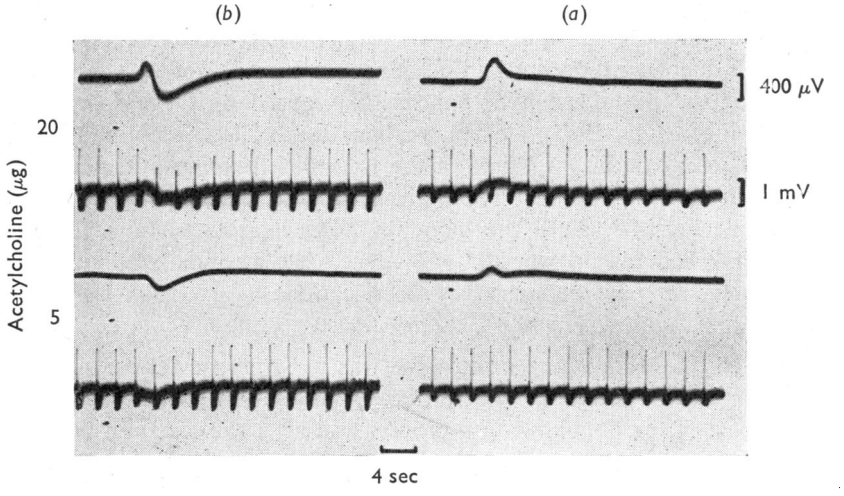

Fig. 4. Ganglion block and potentials produced by acetylcholine before (*b*, left) and after (*a*, right) the administration of 1 μg of atropine. The top tracing of each pair shows the ganglion potentials of the unstimulated ganglion evoked by 5 or 20 μg of acetylcholine before and after atropine. The bottom tracing of each pair shows the effects on the stimulated ganglion of 5 or 20 μg of acetylcholine before and after atropine. (The change in the positive potential following each spike that occurred after the administration of atropine was an inconstant finding.) The vertical calibrations apply to the unstimulated (top) and stimulated (bottom) ganglion respectively.

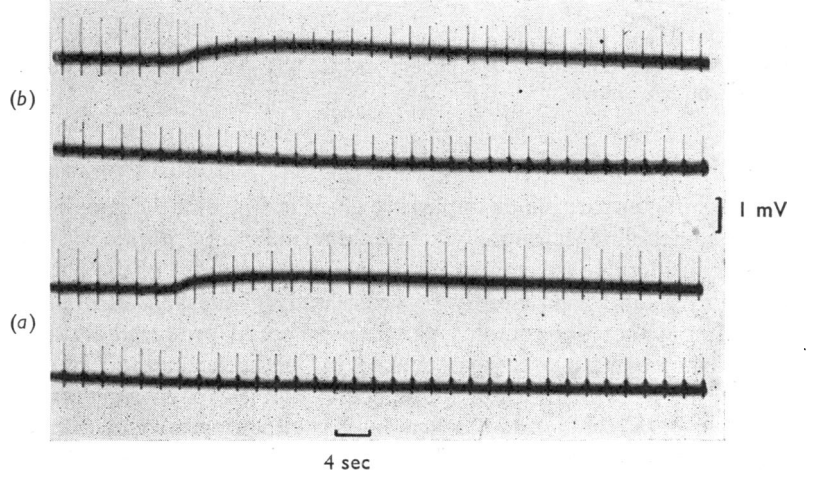

Fig. 5. Antagonism by atropine (1 μg) of the ganglion block produced by tetramethylammonium (12 μg). The top pair of records (*b*) are continuous and show the effects of tetramethylammonium on a ganglion stimulated at a frequency of 0.5 shocks/sec before the administration of atropine. The bottom pair of records (*a*) are also continuous and show the ganglion response to tetramethylammonium after the administration of atropine.

depolarization and the period of sustained hyperpolarization which sometimes followed. Small doses of atropine (0.5 to 2.0 μg) antagonized the ganglion block coinciding with depolarization but had no effect on the block of transmission which occurred during the prolonged hyperpolarization caused by these doses of acetylcholine (Takeshige *et al.*, 1963). When smaller doses of acetylcholine (5 to 50 μg) were used, the reduction in amplitude of the transmitted action potentials occurred during the falling phase of depolarization and during the period of hyperpolarization which followed (Fig. 4). Both the ganglion hyperpolarization and the ganglion block were abolished by atropine (1 to 2 μg). After atropine, the amplitudes of the action potentials were enhanced by acetylcholine.

Atropine antagonized in part the block of transmission produced by tetramethylammonium (Fig. 5). This action of atropine was most prominent during the initial portion of the negative potential. The block of transmission which occurred during the terminal part of the depolarization process and after the ganglion potential had returned to the baseline was unaffected or enhanced by the small doses of atropine. As indicated above, the doses of atropine used did not alter the pattern of ganglion depolarization evoked by tetramethylammonium.

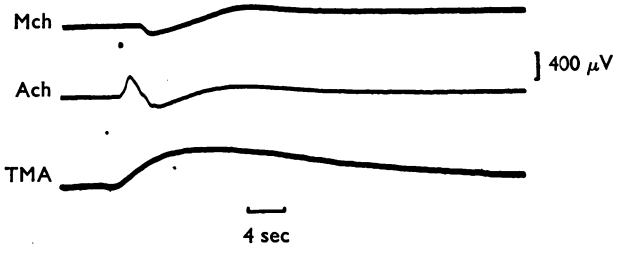

Fig. 6. A comparison of the ganglion potentials evoked by methacholine (Mch, 10 μg), acetylcholine (Ach, 10 μg) and tetramethylammonium (TMA, 1 μg). The records were obtained in three different experiments.

DISCUSSION

For purposes of comparison, examples are given in Fig. 6 of the ganglion potentials evoked by methacholine, tetramethylammonium and acetylcholine. These records illustrate the considerable differences in the ganglionic actions of the cholinomimetic agents used in this and a previously reported study (Takeshige *et al.*, 1963). It is interesting to note that the complex ganglion potential produced by acetylcholine has characteristics common to those evoked by methacholine and by tetramethylammonium. Like tetramethylammonium, acetylcholine evoked ganglion depolarization which was rapid in onset, blocked by hexamethonium, and not blocked by small doses of atropine. In addition, however, acetylcholine produced ganglion hyperpolarization and depolarization which were similar to those produced by methacholine in that they were delayed in onset, blocked by atropine, and either unaffected or increased by hexamethonium. Except for the persistency of the depolarization produced by tetramethylammonium, a combination of the two potentials evoked by methacholine and the one potential produced by tetramethyl-

ammonium would resemble closely the triphasic potential caused by moderate doses of acetylcholine.

It is also noteworthy that this triphasic pattern of potentials bears a striking similarity to the complex ganglion potentials evoked by preganglionic stimulation in curarized rabbit sympathetic ganglia (Laporte & Lorente de Nó, 1950 ; Eccles, 1952 ; Eccles & Libet, 1961). Furthermore, the changes in the contour of the potentials following treatment of the ganglia with hexamethonium or with atropine are in accord with the suggestion that three pharmacologically different cholinoceptive sites, two excitatory and one inhibitory, are present in sympathetic ganglia (Eccles & Libet, 1961 ; Takeshige *et al.*, 1963).

That interrelationships exist among the three cholinoceptive sites is indicated by the alteration in the pattern of the ganglion potential that occurred as the dose of acetylcholine administered was increased. Since large doses of acetylcholine produced an atropine-sensitive ganglion hyperpolarization only in the presence of hexamethonium, it appears that the initial depolarization obscured all evidence of hyperpolarization. Conversely, the block by atropine of the hyperpolarization produced by small doses of acetylcholine unmasked an initial period of depolarization and similarly prolonged the decay-time of the initial depolarization produced by somewhat larger doses of acetylcholine. Accordingly, it is not possible to determine with any great precision either the beginning or the end of the several components of the ganglion potential.

In view of these interrelationships and those existing between the ganglion potentials and the transmitted action potentials, it is tempting to speculate that the periods of ganglion hyperpolarization and delayed depolarization are manifestations of modulating systems in the ganglia. Since the block of transmission produced by moderate doses of acetylcholine coincided with the falling phase of the initial depolarization or with the succeeding period of hyperpolarization, it can be related to the activation of an inhibitory process. Furthermore, the unmasking by hexamethonium of ganglion hyperpolarization in response to larger doses of acetylcholine suggests that the ganglion block produced by these doses of acetylcholine was not related to depolarization but to some inhibitory process. Similarly, the antagonism by atropine of the ganglion block occurring during depolarization by acetylcholine can be attributed to the inhibition of the inhibitory process. On the other hand, the enhancement of the amplitudes of the action potentials which occurred during the delayed depolarization produced either by methacholine or by acetylcholine suggests a facilitatory role in transmission for the receptors giving rise to the delayed depolarization. Thus, it seems reasonable to suggest that the final response of the ganglion to an incoming impulse will be determined to a considerable extent by the existing balance of inhibitory and facilitatory processes.

The nature of the mechanisms underlying the ganglion hyperpolarization resulting from applied acetylcholine and methacholine remains to be determined. Eccles & Libet (1961) have presented evidence which implicates the participation of catechol amines in the process. Lundberg (1952) found that adrenaline produced an inconstant hyperpolarization of the cat superior cervical ganglion which was not always related to block of synaptic transmission. On the other hand, Tauc &

Gerschenfeld (1962) found that some cells of the abdominal ganglion of *Aplysia depilans* responded to iontophoretically applied acetylcholine by depolarization and accelerated rate of spontaneous firing, and that others responded to acetylcholine by hyperpolarization and suppression of firing. Because of the manner of drug application, they concluded that interneuronal mechanisms were not involved in the observed responses, and that the inhibitory response was due to a direct action of acetylcholine on the ganglion cell. In this regard, it is pertinent that cardiac inhibition activated by acetylcholine is associated with hyperpolarization of pacemaker tissues, and does not appear to involve intermediary steps (see Hutter, 1957). Parenthetically, the similarity between the block by atropine of the ganglion hyperpolarization produced by acetylcholine or by methacholine and the actions of atropine on the heart suggests that the former may be a suitable model for the study of peripheral cholinergic inhibitory junctions.

The ganglion block produced by tetramethylammonium does not accord with the forementioned possible mechanisms. Since there was no indication of ganglion hyperpolarization in response to tetramethylammonium either before or after the administration of hexamethonium, and atropine had no effect on the depolarization produced by tetramethylammonium, mechanisms other than the inhibitory processes considered above must be involved. However, the observation that small doses of atropine prevented the block of transmission caused by tetramethylammonium indicates that the block was not necessarily related to ganglion depolarization. As pointed out by Takeshige *et al.* (1963), the limitations of the recording techniques are such that depolarization block by these compounds cannot be eliminated with certainty. It appears, however, that depolarization block occurs only with very large doses of the acetylcholine-like agents. In this connexion, Tauc & Gerschenfeld (1962) observed depolarization block by acetylcholine in *Aplysia* ganglia only after the application of an anticholinesterase agent.

Finally, another form of ganglion block by acetylcholine (Takeshige *et al.*, 1963) and tetramethylammonium has been observed. Following the administration of extremely large doses of acetylcholine, the depression of transmission was associated with ganglion hyperpolarization and continued for some time after the ganglion potential had returned to the control level. Neither the block of transmission nor the ganglion hyperpolarization was relieved by previous treatment of the ganglion with atropine. Similarly, atropine had no effect either on the block of transmission which occurred during the waning seconds of the ganglion depolarization evoked by relatively large doses of tetramethylammonium or on the block of transmission which persisted after the depolarization had dissipated. The delayed depression of transmission may be a reflection of a competitive component in the actions of acetylcholine and tetramethylammonium similar to that proposed for nicotine by Paton & Perry (1953) and Lundberg & Thesleff (1953).

Thus, the characteristics of the block of ganglion transmission produced by the acetylcholine-like agents depends not only on the type of agent used, but also on the amount administered. On the basis of the evoked ganglion potentials and antagonism by atropine, the several forms of ganglion block can be classified as follows: (1) depolarization, antagonized by atropine, produced by acetylcholine or

tetramethylammonium ; (2) hyperpolarization, antagonized by atropine, produced by acetylcholine or methacholine ; (3) hyperpolarization, not antagonized by atropine, produced by large doses of acetylcholine ; and (4) following depolarization, not antagonized by atropine, produced by large doses of tetramethylammonium.

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