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SLOW SYNAPTIC RESPONSES AND EXCITATORY CHANGES IN SYMPATHETIC GANGLIA

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A surface-positive (P) as well as a long-lasting, surface-negative (the late negative, LN) synaptic potential are recorded following suitable orthodromic activation of curarized sympathetic ganglia (Laporte & Lorente de Nó, 1950; Eccles, 1952*a*, *b*; Eccles & Libet, 1961). The probable post-synaptic nature of the responses, and their independence from each other and from the initial negative potential (N), has been established, and a theoretical schema of their origins has been proposed (Eccles & Libet, 1961). The question arose as to whether such slow synaptic potentials can be demonstrated in normally transmitting, i.e. uncurarized ganglia, and, if so, what significance they may have for the course of the excitability state of the ganglion cells following the arrival of preganglionic impulses.

A mode of attacking these questions was made available by the discovery that in curarized ganglia the LN potential (and to a lesser extent P) could be blocked by atropine, and that P (and tc a lesser extent LN) could be blocked by dibenamine (Eccles & Libet, 1961). As such blockade could be accomplished by concentrations of the drugs which had little or no effect on the initial excitatory post-synaptic potential (EPSP, i.e. the surface N potential) it should be possible to block selectively the slow synaptic potentials in uncurarized ganglia, without directly affecting the spike discharge to which the EPSP normally gives rise. If the slow synaptic potentials are elicited in normal ganglia they would contribute to the long-lasting ganglionic after-potentials and changes in excitability which follow orthodromically initiated discharges of the ganglion cells. Such contributions should be eliminated selectively by suitable doses of atropine or dibenamine, leaving behind the 'true' after-potentials which are a direct consequence of spike discharge in the cells, providing that these drugs do not also affect the true after-potentials. In practice, it was not possible to establish clearly the contribution of any P synaptic potential to the total after-potential. The contribution of the LN potential to the after-potentials, however, could be demonstrated quite clearly.

In addition, an analysis was made of the relation of the LN response to the well-known, long-lasting post-tetanic potentiation (PTP) in sympathetic ganglia as described by Larrabee & Bronk (1947). For this purpose the stellate ganglion of the cat with its separable preganglionic inputs was employed. It will be seen that there are in fact two independent types of long-lasting post-activation potentiations; one of these is apparently post- rather than pre-synaptic in nature, it is blocked by atropine, and it corresponds to the LN synaptic potential. Preliminary reports of this work have been published (Libet, 1962*a*, *b*).

METHODS

Preparations and electrodes. For most of the experiments the superior cervical ganglion of the rabbit was used but other ganglia were also studied, especially the stellate ganglion of the cat. All the types of experimental tests were carried out both on isolated ganglion preparations and *in situ* on the blood-circulated ganglion. The procedure for studying the preparation *in vitro* was essentially similar to that already described (Eccles & Libet, 1961). In each isolated preparation the portion of the sheath which encapsulates the ganglion and the proximal region of the post-ganglionic nerve was completely stripped away. Ganglia were kept at room temperature during the period of cleaning and mounting on the electrodes. Under these conditions the superior cervical ganglion of the rabbit gave good responses for many hours; the larger ganglia (cat's stellate) deteriorated at a faster rate than did the rabbit's superior cervical, especially after being warmed up to 37–38° C in the isolated state. The K^+ concentration in the Krebs–Ringer's solution used with rabbit's ganglia in most of this work was lowered to 4 mM, from the previous value of 5 mM, to bring it closer to the concentrations found in rabbit's plasma (*Handb. Biol. Data*, 1956). The bathing fluid was bubbled with 5 % CO_2–95 % O_2 at all times.

The *in vivo* experiments were performed with the animals under light urethane anaesthesia, since this had been found to be relatively innocuous in its effects on synaptic transmission in sympathetic ganglia (Larrabee & Holaday, 1952). The cranial pole of the superior cervical ganglion with the internal carotid division of the post-ganglionic nerve were freed from their attachments without disrupting the blood supply to the ganglion, but they cannot be freed of closely adhering connective tissue in this situation. The mineral oil covering the tissues was heated to 36–38° C with an electrical resistance element immersed in it. Great care must be taken with rabbits not to disturb the rather flimsy connective tissues in the lower region of the neck. If they are disturbed the oil in the pool slowly finds its way to and across the pleural membranes into the intrapleural space; over some hours the animal gradually asphyxiates and dies. A hybrid strain descended from a cross between Flemish Giant and Belgian hare, as well as pure-bred Belgian hares, were found generally to be superior to the more usual laboratory rabbits (New Zealand whites, etc.) in resisting circulatory shock during long experiments; the Flemish Giant hybrid was used for most of the experiments. The internal carotid post-ganglionic nerve is generally longer in the hybrid Flemish Giant than in New Zealand whites, etc., and it is always longer in pure Belgian hares (a fact already noted by Bishop & Heinbecker, 1932).

With the stellate ganglion in the cat, the white ramus of spinal nerve T-3 or T-4, and a length of the thoracic sympathetic trunk below T-4, were freed and mounted on separate pairs of stimulating electrodes. The inferior cardiac nerve and the pole of the stellate ganglion from which it arises were also freed from surrounding tissues without disturbing the vascular connexions of the ganglion. The active ganglionic lead partly encircled this pole, and the active post-ganglionic lead was placed from 4 to 14 mm away on the cardiac nerve. A pool of heated mineral oil covered the area.

The recording leads were chlorided silver wires, 0·3 or 0·4 mm in diameter. All responses were recorded with a dual beam DC oscilloscope after amplification by DC amplifiers (usually used with a high cut-off at 4 kc/s). Ganglionic and post-ganglionic nerve potentials were referred to a common indifferent electrode located on the crushed end of the nerve. For the superior cervical ganglion the active post-ganglionic lead was situated on the nerve two or more millimetres distant from the ganglion. For antidromic responses supramaximal stimuli were applied at the distal end of the post-ganglionic nerve, and recordings were made between one lead on the ganglion and the second on the post-ganglionic nerve some millimetres from the ganglion.

Excitability tests. Changes in level of excitability of ganglion cells, following preganglionic activation, were estimated by delivering a single test preganglionic volley at a set time interval after the end of the conditioning volley or train. For test intervals below 10 sec only one test shock was applied after each conditioning train, so as to avoid influences of one test response on the next. (Values measured are those of the initial S_a spike, even when a test shock strong enough to generate the higher threshold, S_b, component was used.) The changes in amplitude of the test spike are regarded as an index of proportional changes in the number of ganglion cells discharging in response to the same single preganglionic volley. As a relatively large after-hyperpolarization occurs in ganglion cells following the conditioning spike discharge, this could itself raise the amplitude of the test ganglionic spike when superimposed on this after-potential. Therefore, the spike response of the axons, as recorded at the post-ganglionic nerve, was used in plotting excitability. A difference in the recovery course of the post-tetanic test spikes, ganglionic against post-ganglionic, can in

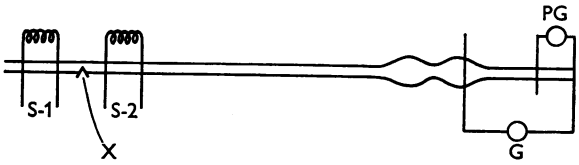

Fig. 1. Diagram of electrode positions on superior cervical ganglion. Recording electrodes, G, with active lead on the cephalic end of the ganglion, and PG, with active lead on the post-ganglionic nerve, have a common indifferent lead on the crushed end of the nerve. Stimulus electrodes S-1 are caudal to point X, at which an incision has partially transected the preganglionic nerve. Electrodes S-2 stimulate the whole cervical sympathetic nerve.

fact be seen by inspection of Fig. 8 and can be accounted for on the basis of the difference in the after-hyperpolarizations. When the post-ganglionic nerve also exhibited a rather large post-tetanic positivity, this complication could not be avoided; however, this post-tetanic positivity was generally unaffected by the drugs tested, and so it would produce a constant error in recovery-of-excitability curves before and during drug action.

Stimuli applied to the preganglionic nerve were of supramaximal strength, both for conditioning and test volleys, in order to avoid complications of small changes in thresholds. A maximal volley in the whole preganglionic nerve (using stimulus point S-2, Fig. 1) does not ordinarily discharge all the ganglion cells, at least under the conditions of these experiments. Excitability curves were also determined, however, with supramaximal stimulation of only a portion of the preganglionic input; this was done by interposing a partial transection, of a third to a half of the width of the preganglionic nerve, between the ganglion and the point of application of supramaximal stimuli (stimulus point S-1, Fig. 1). The results were qualitatively similar with both stimulus locations, although the relative increase in post-tetanic excitability was often greater when S-1 was used.

In any case, no claim is being made that the curves of recovery of test spikes as measured here represent the actual recovery course of excitability level of the ganglion cells in an absolute way. The objective was mainly to compare such curves before and during the action of drugs known to block the slow synaptic potentials, and to relate to the latter any *differences* in the curves. Even so, conclusions about the quantitative influence of a drug on the recovery-of-excitability curve must take into account any changes in the general spike amplitude after administering the drug. A rise in the general spike response, for example, could mean that a greater fraction of ganglion cells fires and is then in the subnormal excitatory state after the response. Since the maximal increase in test spike amplitude that is possible would then be more limited, a curve of post-tetanic test spikes could show an apparent decrease in excitability during such a period with the drug, even when no decrease in an independent post-tetanic facilitatory process had taken place.

RESULTS

After-potentials and slow synaptic potentials

The ganglionic recording of the superior cervical response to preganglionic volleys of impulses regularly exhibits a relatively large after-positivity, lasting about 0·5 sec or more following the spike discharge (e.g. Figs. 2, 3; see also Eccles, 1935*b*; Eccles, 1952*a*; Eccles & Libet, 1961). A rise in strength of preganglionic stimulus, from the level of excitation of the B fibres (which elicit the S_a ganglionic spike) to that of excitation of all B and C fibres (S_a plus S_b ganglionic spikes), further enlarges the amplitude of the after-positivity. The ratios of after-positivity to spike height were generally much lower for the stellate ganglion (e.g. Fig. 4). The post-ganglionic nerve of the superior cervical ganglion exhibited only very small after-positivities with single responses (Figs. 2*B*, 3*D*). With trains of responses, the after-positivities at the post-ganglionic nerve usually increased distinctly (Figs. 3, 8), especially after 40/sec trains (Fig. 2*D*). Ganglia that exhibited marked post-ganglionic positivity during and after even low frequency trains, however, were encountered not infrequently, both *in vitro* and *in vivo* (e.g. Fig. 3*A*); even these ganglia, however, usually showed a very small post-ganglionic after-positivity with single responses (Fig. 3*D*).

An even longer-lasting surface-negative wave can usually be detected following the after-positivity of a single ganglionic spike when sufficiently slow sweep speeds and higher amplifications are employed (e.g. Figs. 2*A*, 3*C*, 6*B*). A late negative hump is regularly found to be superimposed on the after-positivity which follows trains of ganglionic spikes at all frequencies (Figs. 2I, 3I, 4I). At the higher frequencies (and often at the lower ones too) this negative hump remains below the base line and divides the after-positivity into an early deeper phase and a longer, shallower tail (e.g. Figs. 2I*D*; 4I*B*, *C*). The negative hump in the after-positivity was seen in all mammalian sympathetic ganglia which have

been examined (superior cervical ganglia of rabbit and cat; coeliac and stellate ganglia of cat) provided that these are in good condition, i.e. either circulated with blood or, when isolated, tested within the first several

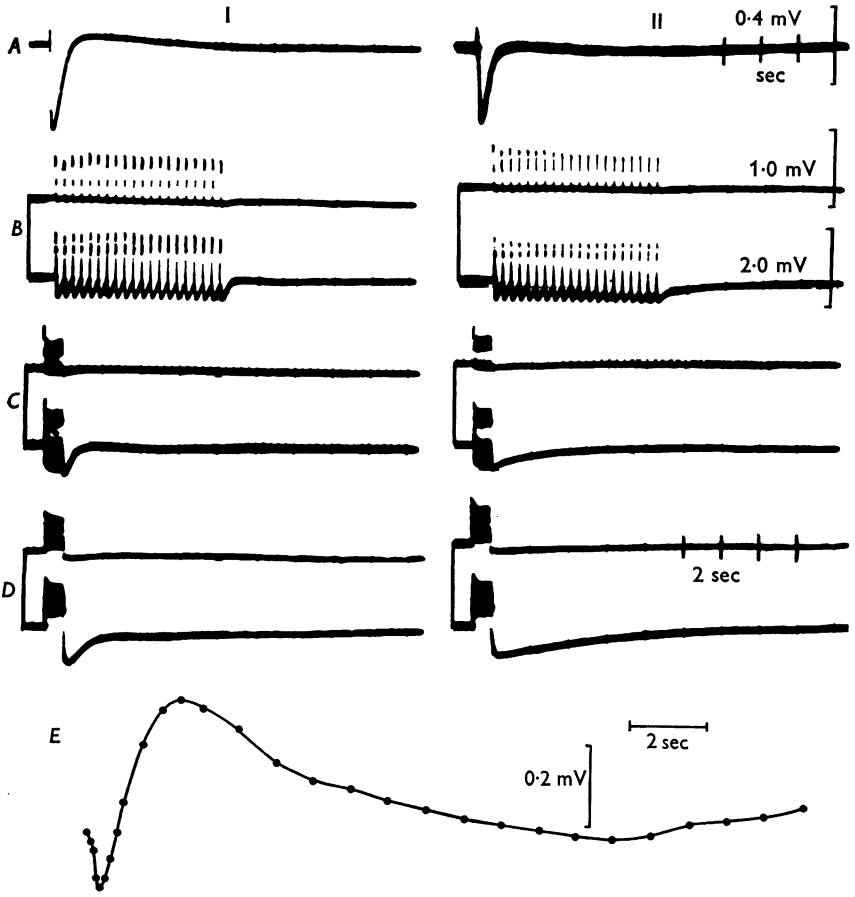

Fig. 2. Effect of atropine on ganglionic discharge and 'after-potentials', *in situ*. Superior cervical ganglion of rabbit, stimuli at S-2 supramaximal for B and C fibres. For each pair (in *B*, *C* and *D*) the top tracing gives the responses of the post-ganglionic nerve, the bottom tracing gives the responses at the ganglion. (This will be true also for all other such paired tracings in other figures, except for Fig. 4.) Vertical column I responses before atropine, column II after administering atropine (0·5 mg/kg I.V.). *A*, single ganglionic response at higher gain (action potential up, off screen), showing the after-positivity and small late negativity following it; *B*, responses to a 2/sec train of stimuli lasting 10 sec; *C*, 10/sec, 1 sec train; *D*, 40/sec, 1 sec train. *E*, plot of the measured difference between ganglionic after-potentials in *D* I and *D* II (with voltage and sweep speed scales magnified as shown). Calibrations shown in *B* II and *D* II apply to all *B*, *C*, *D*. Polarity is negative upwards for the active leads, in this and all other figures. Faint spikes have been retouched in all figures.

hours after warming to 37° C. The after-positivity recorded at the post-ganglionic nerve possessed little or no such negative inflexion.

Atropine. In low concentrations, similar to those which could block the LN synaptic potential in curarized ganglia (Eccles & Libet, 1961), atropine consistently and markedly reduced or eliminated the late negative hump from the after-potentials which follow ganglionic spike discharge that has been elicited orthodromically. This occurred both with isolated ganglia, at concentrations of 0·1–0·2 μg/ml. of atropine sulphate (e.g. Figs. 3 and 4), and with ganglia through which blood circulated *in situ*

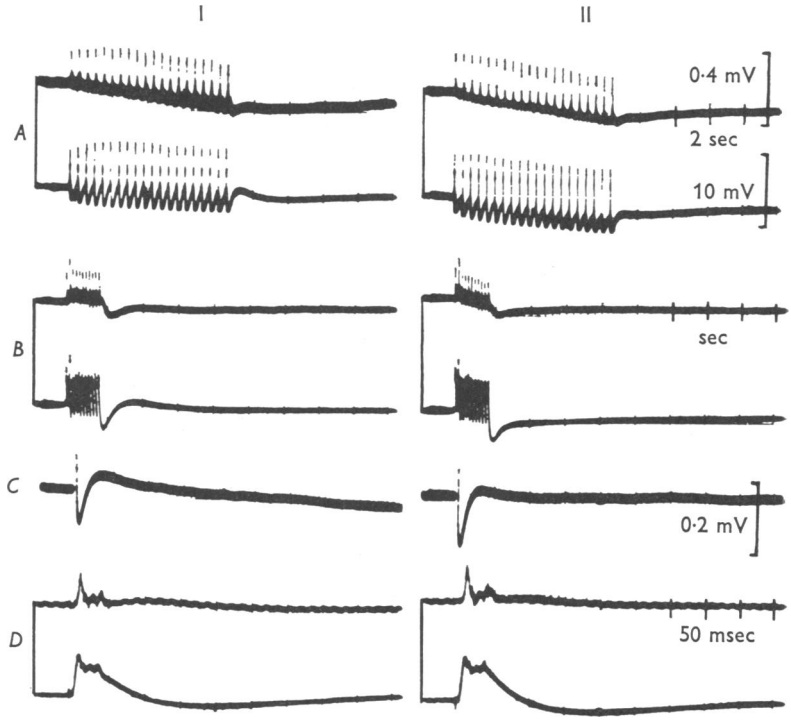

Fig. 3. Effect of atropine on ganglionic responses, *in vitro*. Superior cervical ganglion of rabbit, stimuli at S-1 supramaximal for B and C fibres (giving about 50 % of the ganglionic spike height obtainable with S-2). Column I before, Column II with atropine (0·2 μg/ml. in bath). *A*, 2/sec, 10 sec train; *B*, 10/sec, 1 sec train; *C*, single ganglionic 'after-potentials' (action potential up, off screen); *D*, single stimulus. Voltage calibrations in *A* II hold for *A*, *B*, *D*; in *C* II, for *C* only. Time calibration in *A* II for *A*; *B* II for *B* and *C*; *D* II for *D*.

(e.g. Fig. 2). With the latter, doses of 0·5 mg/kg I.V. were generally used. No attempt was made to determine whether this was a minimum dose requirement for this change in after-potentials (but, in one rabbit, a dose of atropine 0·2 mg/kg eliminated a large fraction of the late negative hump).

When a single ganglionic response and its after-positivity was followed by a small late negative wave, the latter was also greatly reduced by atropine (Figs. 2*A*, 3*C*, 6*B*). The virtual elimination of the late negative hump by atropine leaves behind a long-lasting after-positivity of a simpler form, one which now appears to decay exponentially (Fig. 2II*B*, *C*, *D*).

The ganglionic after-potentials elicited in the stellate ganglion were small. Even so, at suitable amplifications similar changes in the form of the after-potentials upon administering atropine could be demonstrated clearly (Fig. 4). In Fig. 4III are also shown the ganglionic potentials of the curarized contralateral stellate ganglion from the same cat as for Fig. 4I and II. The LN potential which follows the summed EPSPs (N potential) that appear during each train of stimuli was completely

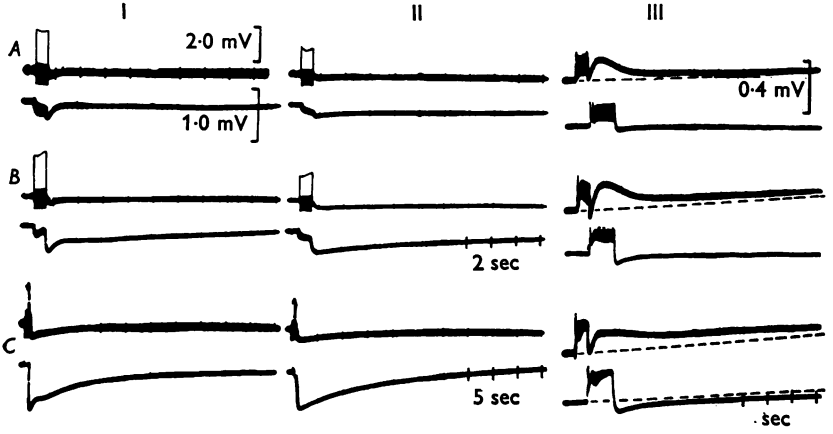

Fig. 4. Effect of atropine on stellate ganglion responses. Stellate ganglion of cat, *in vitro*, supramaximal stimuli at the thoracic sympathetic chain below ramus T-4. Column I before, column II with atropine (0·1 μg/ml.); column III, contralateral stellate ganglion of same animal, ganglion curarized with dihydro-β-erythroidine (15 μg/ml. in bath). *A*, 10/sec, 1 sec train; *B*, 20/sec, 1 sec train; *C*, 40/sec, 1 sec train. The bottom tracing of each pair, in columns I and II, is the same as top tracing but taken at about 3 times the amplification (action potentials not shown in the bottom tracings). The bottom tracing of each pair in column III shows responses of the curarized ganglion with atropine (0·1 μg/ml.) also present (sweep speeds inadvertently twice as fast in bottom tracings of column III). Voltage calibrations in *A* I, for *A*, *B*, *C* in columns I and II; calibrations in *A* III, for column III. Time calibration in *B* II, for *A* and *B*, in columns *C* II, for *C* in I and II; calibration in *C* III, for bottom tracings of *A*, *B*, *C*, in III.

wiped out by adding a similar concentration of atropine to the bath (Fig. 4III, lower tracing of each pair) leaving behind a small P synaptic potential, as reported previously (Eccles & Libet, 1961).

The durations and amplitudes of the LN synaptic potentials (e.g. in

Fig. 4III) appear to be comparable (when allowance is made for the differences in amplification) to the late negative humps which are eliminated by atropine from the after-potentials of the uncurarized ganglia (e.g. in Fig. 4I and II). The algebraic difference between the ganglionic after-potentials before and after administering atropine can be plotted as in Fig. 2*E*, and bears a striking resemblance to the LN synaptic potential (perhaps preceded by a fraction of a P synaptic potential which can also be reduced by atropine).

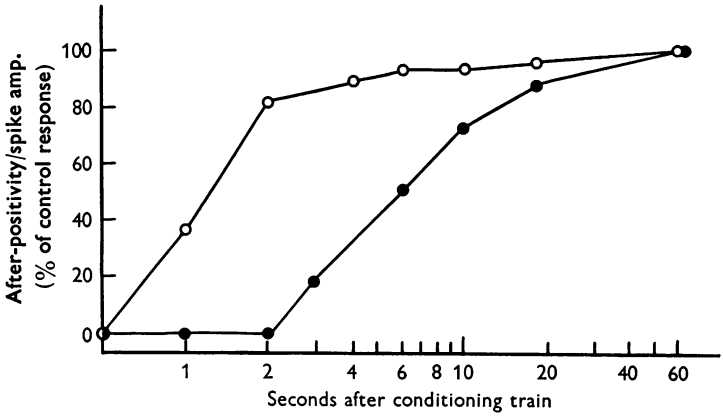

Fig. 5. Effect of post-tetanic 'after-potential' on ganglionic after-positivity of single test responses. Data taken from the experiment shown in Fig. 8. The ratio of the amplitude of after-positivity (in ganglionic record) to that of the action spike (post-ganglionic nerve) is represented on the ordinate as a percentage value, taking that for the pre-tetanic, control single response as 100 %. (This ratio, instead of the recorded amplitude of the after-positivity itself, is used so as to exclude changes in the latter that may be due to any alteration in the number of ganglion cells that have discharged.) Open circles, before atropine; filled circles, with atropine (0·5 mg/kg).

A further piece of evidence is interpretable only on the basis that this LN post-synaptic component, superimposed on the true after-potential, originates in the ganglion cells which are actually discharging the recorded post-ganglionic impulses. This evidence is present in the changes in the after-positive wave which follows each ganglionic test spike when the latter is superimposed on the post-tetanic after-potential, as shown in Fig. 8. The after-positivity of the test spike had a maximum amplitude about 30 % that of the spike in the control response in this case. In Fig. 5 the ratio of the ganglionic *after-positivity* of each single test response relative to its post-ganglionic spike height has been plotted against the post-tetanic time interval. The recovery of the after-positivity of a single response (relative to its test spike) has a time course similar to that of the

post-tetanic after-hyperpolarization. Such a correlation has already been observed in normal, non-atropinized ganglia (Eccles, 1935*b*; Eccles, 1952*a*), and is explained by the occurrence of the single test discharge in ganglion cells whose membranes are already in some state of hyperpolarization. A similar correlation is seen in the atropinized ganglion; i.e. the recovery of the after-positivity of the single response is further delayed after the reduction in LN component by atropine and the prolongation of post-tetanic after-hyperpolarization which results from this. Such change in post-tetanic after-hyperpolarization, however, can only affect the after-positivity of a superimposed test response in those ganglion cells which both undergo this change and respond in the test discharge. It follows, therefore, that the LN potential was blocked or reduced by atropine in the same ganglion cells which gave rise to the after-positivity of the superimposed test ganglionic discharges.

The amplitude of the spike discharge with a single preganglionic volley, at ganglion and post-ganglionic nerve, was not specifically affected by atropine itself. With the *in situ* preparations, spike amplitude often remained steady during an atropine test (e.g. Fig. 2), or it sometimes increased or decreased to some extent. With the *in vitro* preparations spike amplitude often remained unchanged, occasionally increased slightly (e.g. Fig. 3*D*), and more often decreased to some extent in accordance with the slow progressive deterioration of responses which occurs *in vitro*. Such deterioration of the isolated ganglia with time was usually more rapid with the larger ganglia like the stellate, and undoubtedly accounts for the rather large decrease in spike amplitude which is seen in Fig. 4 following addition of atropine.

Dibenamine and reserpine. With isolated uncurarized ganglia the LN hump was only partially reduced by dibenamine (1–5 μg/ml.), in contrast to the effect of atropine. This was also observed *in vivo*.

A reduction in amplitude of the after-positivity of single ganglionic spikes often followed the administration of dibenamine or reserpine; this is as predicted from the postulate of an adrenergic step in the origin of the P synaptic potential (Eccles & Libet, 1961). This occurred in spite of a frequently striking increase in amplitude of ganglionic and post-ganglionic spikes. The dose of dibenamine was 8–15 mg/kg I.V., which, after 1–2 hr, largely but never entirely blocked the ability of a test injection of about 20 μg of adrenaline to depress markedly the post-ganglionic spike response to a preganglionic volley. With reserpine a single dose of 1·25 mg/kg I.V. was given, and responses were followed for 7–8 hr thereafter (Muscholl & Vogt, 1958; Costa, Revzin, Kuntzman, Spector & Brodie, 1961).

Control periods without any drugs, however, frequently exhibited similar increases in post-ganglionic spike heights and relative reductions in ganglionic after-positivity. Also, a clear-cut lengthy delay of 5–7 hr was not required for these changes to appear after administering reserpine, although the largest changes did occur after some hours. The impression was gained that changes in control ganglionic responses were associated with those periods during which mean arterial pressure had remained at a low level (30–40 mm Hg) for some time, and/or the level of arterial oxygenation had appeared to be chronically low. As is

well known, rabbits are liable to exhibit such poor circulatory conditions during long surgical or experimental procedures. Since the administration of dibenamine or reserpine led sooner or later, respectively, to a fall in arterial pressure to similar low levels of 30–40 mm Hg, it is obviously possible that this factor operated in every instance after administering these drugs. With the present data it is not possible to tell in any statistically valid manner whether the changes with the drugs quantitatively exceeded those during control periods.

Antidromic and axonal after-potentials. Ganglionic responses to antidromic impulses, as well as responses purely of post-ganglionic axons, should be free of any LN or P potentials if these are indeed synaptically generated. Valid quantitative comparisons between the ganglionic after-positivities of orthodromic against antidromic responses, however, cannot be made. Considerably fewer than 100 % of the ganglion cells fire in response to a maximal orthodromic volley, judging from the post-tetanic increases in such responses (see below). Similarly, it seemed clear that antidromic volleys were not invading all the ganglion cells. (For this purpose the amplitude and area of the ganglionic after-positivity, i.e. of the larger after-hyperpolarization in the region of the soma, were taken to be indices of the relative number of cell bodies fired antidromically. It was possible in a lightly curarized ganglion to increase considerably the ganglionic after-positivity of antidromic responses by coupling the arrival of the antidromic impulses with that of a submaximal orthodromic volley; the latter by itself produced only an EPSP which facilitated antidromic invasion, and any P synaptic potential that it elicited was subtracted from the combined total.) Obviously there can be no assurance here that the same number of ganglionic cells respond to a maximal orthodromic as to a maximal antidromic volley, and that the true after-hyperpolarizations contribute identical portions of the after-positivity in both cases.

Some points of interest remain, however:the ganglionic after-positivity following an antidromic tetanus did not exhibit an LN hump; nor was the latter present in the after-positivity of trains of impulses in isolated post-ganglionic nerve (inferior cardiac nerve separated from the stellate ganglion). Atropine (0·1–0·2 μg/ml.) did not modify the shape of these after-positivities, in contrast to its effect on orthodromic responses. The amplitude of the ganglionic after-positivity with antidromic activation was not depressed by dibenamine (2 μg/ml.). Nor was any selective effect by dibenamine or atropine seen on the after-hyperpolarization potentials of the post-ganglionic axons (isolated inferior cardiac nerve).

Post-ganglionic discharge during a train of responses

As the LN synaptic potential can be seen to build up during a train of repetitive preganglionic volleys in curarized ganglia (Eccles & Libet, 1961), its presumed excitatory effect should have some progressive influences on

the number of discharging ganglion cells during a train of responses. By selectively depressing the LN component the difference that this slow potential makes in the responses should become manifest.

The influence of atropine on the size of the post-ganglionic responses during a train of maximal preganglionic volleys was more evident with trains of low frequency, 2 or 10/sec, than with higher frequencies. This is so presumably because the LN synaptic potential builds up at these lower frequencies relatively more effectively than either the P synaptic potential (Eccles & Libet, 1961) or the true after-hyperpolarization; a purer LN effect can thus by studied by using low frequency stimuli. The depression by atropine of an apparent LN synaptic potential during a train may be

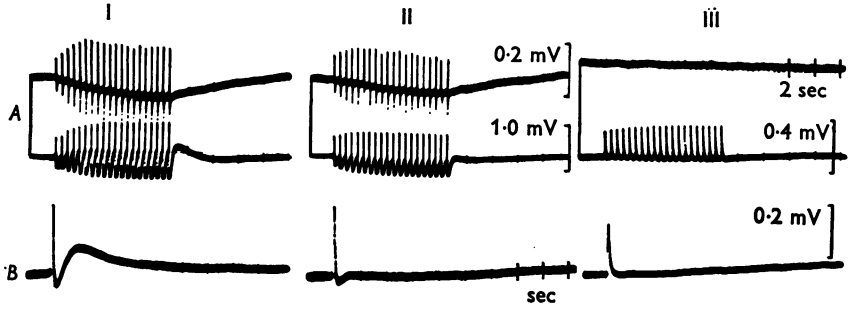

Fig. 6. Responses during a low frequency train; with synaptic transmission depressed. Superior cervical ganglion, of rabbit, *in vitro*, opposite side to ganglion used in Fig. 3; stimuli at S-2, supramaximal for B and C fibres. Column I, in presence of weakly curarizing concentration of dihydro-β-erythroidine (7·5 μg/ml.); column II, after addition of atropine (0·2 μg/ml.) to same bath; column III, after raising concentration of the dihydro-β-erythroidine to 17·5 μg/ml. in same bath (note that spike discharge is now blocked completely, only ganglionic EPSPs remaining). *A*, 2/sec, 10 sec train; *B*, single response (ganglionic record only) and at higher gain. Voltage calibration on top tracing of *A* II applied to all top tracings on *A*; in bottom of *A* II applied to *A* I and *A* II bottom; on *B* III applies to all *B* tracings. Time calibration on *A* III applies to all *A*, on *B* II to all *B*.

seen in Fig. 3*A*, and less so in Fig. 2*B*. This was accompanied by a change in the progress of the amplitude of the spike recorded at post-ganglionic nerve. Qualitatively similar effects in the post-ganglionic discharge may also be seen during a 10/sec train, as in Fig. 3*B*.

Recruitment was especially prominent when ganglion cells were depressed (see also Bronk, Tower, Solandt & Larrabee, 1938). In ganglia which have been partially curarized so that only a small spike remained, repetition of preganglionic volleys at low frequencies (such as 2/sec) could greatly build up the amplitude of the post-ganglionic spike responses (e.g. Fig. 6I*A*). When atropine was added the LN potential which formerly built up during the train was virtually eliminated (Fig. 6II*A*,

B) and the recruitment of post-ganglionic spike discharge could be greatly reduced (e.g. Fig. 6II*A*). Such an effect of atropine on recruitment was observed in the stellate as well as in the superior cervical ganglia. These effects of atropine indicate that the build-up in the LN synaptic potential could in fact be a direct cause of the recruitment of post-ganglionic discharge. The findings of Takeshige & Volle (1962), that the asynchronous post-ganglionic discharge elicited during trains of stimuli to the preganglionic nerve of curarized ganglia could be blocked by atropine, are also explained by this mechanism.

While atropine was able to eliminate most of the recruiting effect of low frequency repetition in some ganglia (as in Fig. 6), in others it reduced this effect only fractionally or hardly at all, even though the LN potential was depressed by it. Fig. 6III demonstrates, further, that some progressive rise in the initial or fast EPSP can occur with repetition at 2/sec even in the apparent absence of any LN response. It would appear, therefore, that an atropine-resistant facilitatory process can also participate to some extent in such recruitment even with intervals as long as 500 msec between stimuli; this presumably involves 'presynaptic PTP' (see below), since the initial fast EPSP has a total duration of less than 50 msec when recorded intracellularly (Eccles, 1955, 1963).

Recovery of excitability after orthodromic activation

Homosynaptic testing of superior cervical ganglion. Long-lasting potentiations of the post-ganglionic test spike were seen following preganglionic conditioning trains of 1 sec duration and frequencies of 10 and 40/sec, although there was an initial period of depression lasting a fraction of a second. Actual recordings of the responses upon which such excitability curves are based are shown in Figs. 7 and 8. The recovery-of-excitability curves so determined are shown in Fig. 9. When only a portion of the preganglionic nerve was stimulated (at S-1, Fig. 1) the potentiation was considerably greater, on the basis of percentages, than that obtained by stimulation of the whole nerve (S-2). This is presumably due to the relatively larger fraction of the neurones which are not in a post-discharge phase of subnormal excitability, following maximal stimuli at S-1. Such marked differences between the two types of excitability curves, however, did not occur in all the ganglia so tested.

After a conditioning train of stimuli at 2/sec for 10 sec, the post-ganglionic test spike also exhibited a considerable potentiation (Fig. 10). In such cases the potentiation was preceded by little or no depression; this is in accord with the comparatively small after-positivity in the ganglionic record following such trains (e.g. Fig. 2I*B* and 3I*A*).

The administration of atropine resulted in the elimination of most, and

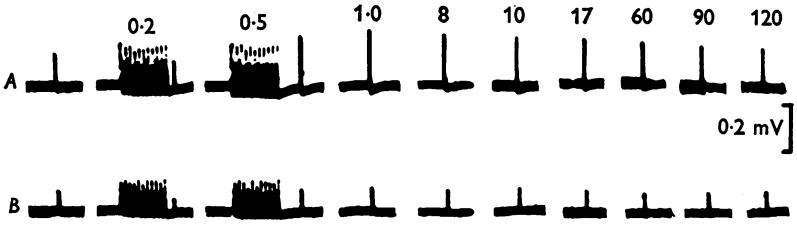

Fig. 7. Test responses after a 10/sec train. Same experiment as in Figs. 2 and 9; only post-ganglionic nerve responses shown here. Conditioning trains, 1 sec duration, supramaximal for B and C fibres, at S-1; test shocks maximal for B fibres only at S-1. Row *A* before, and row *B* after injecting atropine (0·5 mg/kg, I.V.). In each row the initial control, pre-conditioning, test response is followed by a series of separate conditioning trains each with a test response following it by the stated time interval. (The records of the conditioning trains are included here only in the first two of the series. The test responses at 17, 60, 90, and 120 sec intervals all follow the same conditioning train.)

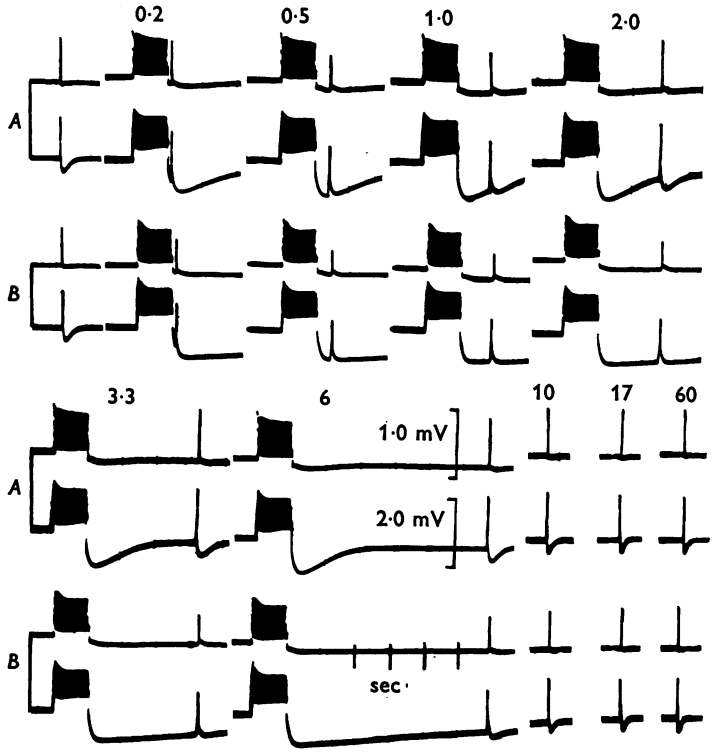

Fig. 8. Test responses after a 40/sec train. Same experiment as in Figs. 2 and 9. Conditions and arrangement similar to that in Fig. 7, except that stimuli are at S-2. (Records of conditioning trains not included for the 10, 17, and 60 sec test intervals.) Time calibration applies to all, except for the 10, 17, and 60 sec responses, which are at one-half this sweep speed.

sometimes all, of the long-lasting potentiation that was seen following all such conditioning trains (Figs. 9, 10). In addition, the initial phase of depression when present was lengthened to a period of some seconds, in accordance with the more prolonged depth of ganglionic after-positivities

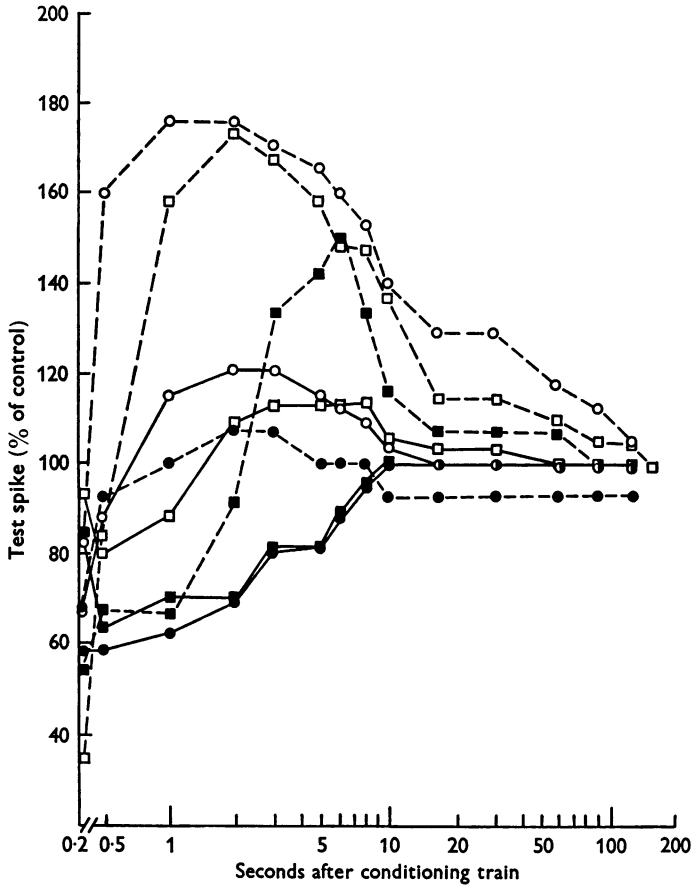

Fig. 9. Recovery of excitability after 1 sec conditioning trains, 10 and 40/sec. Same experiment as in Figs. 2, 7, and 8. Conditioning trains supramaximal for B and C fibres, test shocks maximal for B fibres, both delivered at S-1 (interrupted lines) or at S-2 (continuous lines); circles signify 10/sec trains, squares 40/sec trains. Open symbols are for before, filled symbols are for after the injection of atropine.

that prevail with atropine present (Figs. 2II, 3II, 4II). It thus seems clear that in the superior cervical ganglion the bulk of the long-lasting post-tetanic potentiation that is seen after conditioning trains, at the durations and frequencies tested, can be blocked out by low concentrations

of atropine. The effective doses of atropine which are required *in vivo* for this blockade are probably considerably less than the dose of 0·5 mg/kg I.V. would indicate. About an hour was required after the injection of atropine for carrying out the full series of excitability tests; during this time it is likely that a considerable fraction of the atropine was metabolized in the rabbit (Ambache, 1955).

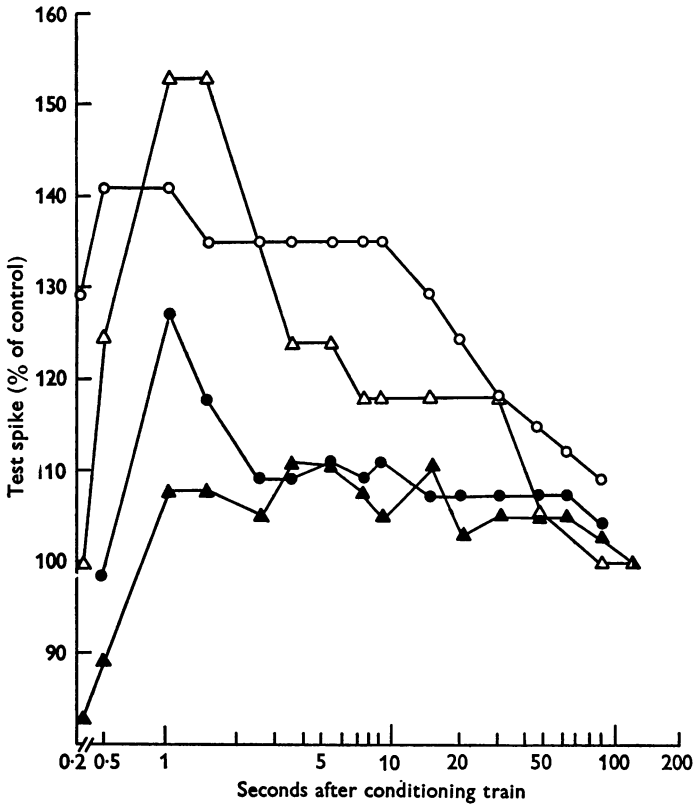

Fig. 10. Recovery of excitability after 10 sec conditioning train at 2/sec. Same ganglion as in Fig. 3. Conditioning trains and test shocks both supramaximal for B and C fibres, both delivered at S-1 (circles) or at S-2 (triangles). Open symbols are for before, filled symbols are for after addition of atropine (0·2 μg/ml.).

Changes in post-activation excitability curves after administration of dibenamine or reserpine were quite variable for the different preparations. A rise in amplitude of the facilitatory phase, which would be expected if these agents selectively removed any P synaptic potential from the post-activation period, did in fact occur in some instances with dibenamine (in spite of an increase in spike amplitude and some reduction in the LN component of the after-positivity). As already noted above, however, any reductions in the true after-hyperpolarization, perhaps because of lowered blood pressure produced by these agents, would affect the excitability curves in the same general way as would loss of P synaptic potential; the interpretation of these results is therefore unsatisfactory.

Heterosynaptic compared with homosynaptic testing. The long-lasting post-activation facilitatory phase described in the present work bears a marked resemblance in some respects to the well-known phenomenon of post-tetanic potentiation (PTP), as described for sympathetic ganglia (Larrabee & Bronk, 1947) and other structures (reviewed by Hughes, 1958). To clarify the relation to PTP it was necessary to determine

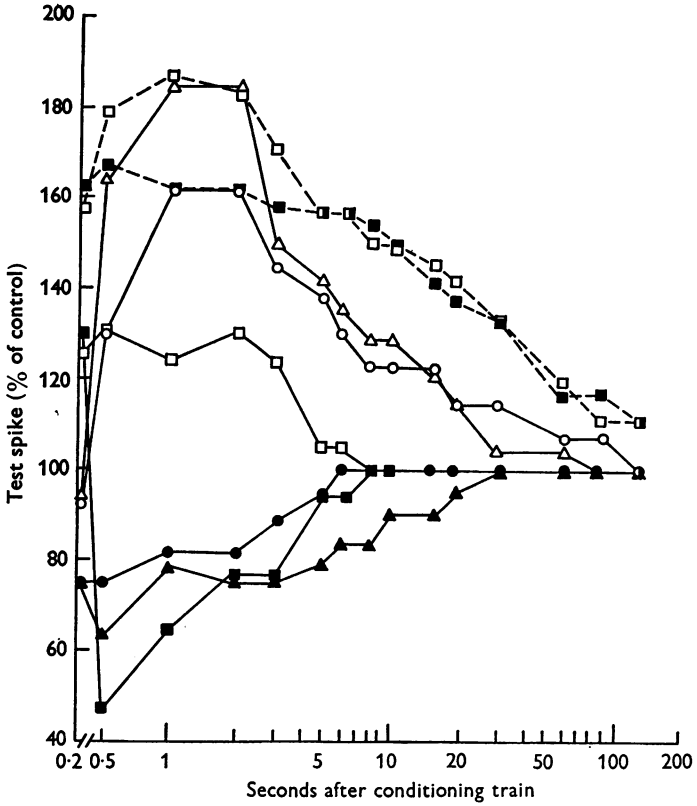

Fig. 11. Heterosynaptic testing of post-tetanic excitability. Stellate ganglion of cat, *in vitro* (same one as in Fig. 4, I and II). Conditioning trains 1 sec duration, at 10/sec (circles), 20/sec (triangles) and 40/sec (squares), supramaximal on the thoracic sympathetic chain below white ramus T-4; test shocks, supramaximal, heterosynaptic at ramus T-4 (continuous lines) or homosynaptic at thoracic chain (interrupted lines). Open symbols before, filled symbols after addition of atropine (0·1 μg/ml.).

whether, in contrast to classical PTP, the present LN facilitation could be demonstrated with heterosynaptic testing; and to determine whether classical PTP is sensitive to atropine blockade in the way that LN facilita-

tion is. In order to examine these points, the stellate ganglion of the cat, with its separate preganglionic input branches, was employed.

A long-lasting post-tetanic facilitation was found even with heterosynaptic testing in the stellate ganglion, both in the isolated state (Fig. 11) and *in situ* (Fig. 12). The form, duration and order of magnitude of heterosynaptic potentiation was roughly similar to that which was exhibited with

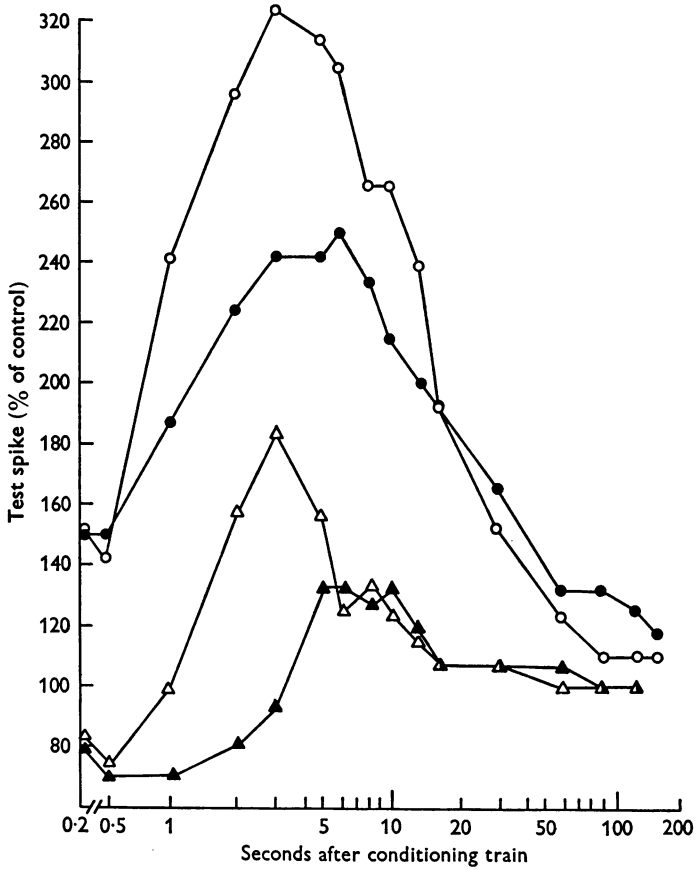

Fig. 12. Heterosynaptic and homosynaptic testing of post-tetanic excitability. Stellate ganglion of cat, *in situ*. Conditioning trains, 1 sec duration, at 40/sec, supramaximal on the thoracic sympathetic chain below ramus T-4; test shocks supramaximal, heterosynaptic on ramus T-3 (triangles) or homosynaptic on thoracic chain (circles) at site of conditioning stimuli. Open symbols for before, filled symbols for after injection of atropine (0·5 mg/kg, I.V.).

homosynaptic testing, in the stellate and superior cervical ganglia, with the frequencies and train durations shown here. Indeed in some isolated ganglia the heterosynaptic PTP exceeded the homosynaptic one. A small,

more variable post-activation potentiation following a single orthodromic volley was also seen with heterosynaptic as well as with homosynaptic testing.

The heterosynaptic PTP was virtually completely abolished by atropine, being replaced by a phase of depression or by enlargement of amplitude and duration of the initial brief depression when this was present (Figs. 11, 12). There is thus no doubt that the atropine-sensitive, LN synaptic response accounted for the heterosynaptic PTP, as it did for the bulk of the homosynaptic PTP in the superior cervical ganglion.

Atropine eliminated only a fraction of the homosynaptic PTP in the stellate ganglion. This fraction was rather small in the isolated preparations tested (e.g. Fig. 11), but this may have been partly a fortuitous result of the experimental procedure.

It was already noted above that the LN potential tends to become smaller and eventually disappear, this beginning some hours after warming to 37° C. Since in a given preparation all of the heterosynaptic tests were routinely carried out as a group earlier than the homosynaptic tests, there is a possibility that a greater loss in the LN portion of PTP had occurred before the homosynaptic testing. In Fig. 12 it is seen that in an *in situ* experiment the amount of atropine-sensitive PTP was roughly comparable for homo- and heterosynaptic testing. More of such *in situ* experiments would have to be conducted to establish clearly the quantity of atropine-sensitive homosynaptic PTP which the stellate ganglion can exhibit. Another point that would require clarification is the degree of constancy of the atropine-resistant portion of the homosynaptic PTP as an experiment progresses; variations in this portion would obviously affect the estimation of the atropine-sensitive portion.

The important comparison established at this time is that a large amount of the homosynaptic PTP is not atropine-sensitive here, while the heterosynaptic PTP is virtually entirely atropine-sensitive. This refers of course to PTP following the kinds of conditioning trains employed here and not necessarily to that exhibited after long trains at high frequencies.

DISCUSSION

Slow excitatory post-synaptic potential. The changes induced by atropine, in the after-positivity, in the recruitment at low frequencies, and in the recovery of excitability following orthodromic ganglionic discharge, leave no doubt that a slow, surface-negative, facilitatory potential, identical with the LN post-synaptic potential exhibited by curarized ganglion, is contributing significantly to the potentials and excitability states in normal (i.e. uncurarized) ganglia. These changes are found at least as well in the intact ganglion through which blood circulated as in the isolated one. The generation of the LN synaptic potential does not, then, depend upon any inability to remove acetylcholine in the normal manner. Evidence was also presented which shows that the LN synaptic potential is generated in the same ganglion cells that discharge the post-ganglionic nerve impulses,

rather than in a separate hypothetical group of cells that generate only these slow potentials. This is further supported by the observation that subthreshold doses of muscarine (which undoubtedly acts at the LN receptors) potentiate the post-ganglionic discharge elicited by a preganglionic stimulus (Sanghvi, Murayama, Smith & Unna, 1963).

It would seem advisable to change the term for the LN synaptic potential to the *slow EPSP* (excitatory post-synaptic potential). The term 'slow' would distinguish this response from the initial, shorter-lasting, better-known EPSP, but the suggested term would keep the uniformity of the usage 'EPSP' for depolarizing post-synaptic responses which raise the level of cell excitability. Slow EPSPs of up to about 25% of pre-curare spike height can be demonstrated after brief preganglionic trains in mildly curarized ganglia (Eccles & Libet, 1961), when there are no after-hyperpolarizing potentials to mask their magnitude. Such a percentage of spike amplitude would not be far different from the firing level of sympathetic ganglion cells (Eccles, 1955, 1963; Nishi & Koketsu, 1960). Since in uncurarized ganglia the slow EPSP overlaps with the period of after-hyperpolarization following spike discharge, this would reduce the possibility of the slow EPSP being sufficient by itself to depolarize the cell to the firing level. At least under certain conditions, however, preganglionic impulses can apparently elicit actual ganglionic discharge through the slow EPSP mechanism; the asynchronous post-ganglionic after-discharge which may follow a suitable preganglionic tetanus was found to be blocked selectively by atropine (Takeshige & Volle, 1962).

An attempt to detect a slow EPSP with intracellular recording has been made, with a negative result (Eccles, 1963): but with sampling by the micro-electrode restricted to only those ganglion cells located at the very surface of the ganglion, the question cannot be considered as settled in view of the convincing evidence from the macro-electrode recordings at the surface. It has been noted here, for example, that the LN potential tends to disappear from the responses of the the isolated ganglion after some hours at 37° C; perhaps this loss is greatly accelerated for the cells at the surface of the desheathed ganglion.

Post-activation potentiations. The evidence presented demonstrates that there are, clearly, two types of long-lasting post-activation potentiations: One is associated with a post-synaptic, cholinergic atropine-sensitive response (the slow EPSP) which can be manifested even with heterosynaptic testing; the other is associated with atropine-resistant changes presumably in the presynaptic terminals, and is only seen with homosynaptic testing (Larrabee & Bronk, 1947; Lloyd, 1949; Eccles, 1957). It should be noted, parenthetically, that blockade by small doses of atropine of a post-activation potentiation represents a clear demon-

stration of the selective sensitivity to atropine of a physiologically significant process in autonomic ganglia.

The actual PTP which can be observed with homosynaptic testing in sympathetic ganglia is a summed effect of both types of potentiations, but the relative contribution of each type probably varies with the location of the ganglion and its condition, and perhaps with the frequency and duration of the conditioning impulses. No systematic study was made of these possible factors, although some differences have already been noted between cat's stellate and rabbit's superior cervical ganglia.

It is difficult to pin-point those differences in experimental conditions which could explain the inability of Larrabee & Bronk (1947) to find any evidence of PTP with heterosynaptic testing, assuming that their animals had not been given any atropine. One stellate ganglion (*in situ*) in the present work also exhibited no heterosynaptic PTP, while good PTP was seen with homosynaptic testing following 40/sec, 1 sec trains, and this homosynaptic PTP was unaffected by atropine. Some possible reasons for failure to elicit heterosynaptic PTP are: (*a*) the LN and P synaptic potentials are both considerably more labile under adverse conditions than is the initial EPSP: (*b*) repeated delivery of long conditioning trains (of 10–15 sec duration) during the course of their experiments could perhaps have produced a prolonged depression of the LN synaptic potential; some preliminary evidence on the LN potential in heavily curarized ganglia indicates that this is a possibility (Libet, unpublished): (*c*) at times, and especially with the use of higher preganglionic frequencies for the conditioning trains, post-tetanic depression may be large and present in a sufficiently high percentage of ganglion cells to mask any facilitation provided by the slow EPSP. (The extra addition of presynaptic PTP may help to overcome this in the homosynaptic tests.) Although the excitability curve would then show little or no net heterosynaptic PTP under these conditions, the administration of atropine might still have revealed that a component of slow EPSP facilitation was in fact contributing to the total excitability pattern. Knowledge of such an atropine effect was of course not available to Larrabee and Bronk. In the work of Job & Lundberg (1953), who failed also to observe heterosynaptic post-activation potentiation, only single conditioning volleys were used; these would be expected to elicit only very small LN potentials.

There is at least one other example reported of PTP which can be elicited by heterosynaptic testing. Wilson (1955) found that the polysynaptic reflex discharge of the spinal cord in response to test volleys in one dorsal rootlet showed PTP after a conditioning tetanus in an adjacent dorsal rootlet. This heterosynaptic PTP was in some cases as great as that found with homosynaptic testing. Wilson preferred to explain this heterosynaptic PTP in terms of presynaptic changes, which were assumed to occur in this case in the presynaptic terminals of second or higher order neurones in the reflex chain. No interneuronal mediation is known to be available in sympathetic ganglia with which to explain the atropine-sensitive PTP found there. The existence of a slow EPSP type of PTP in ganglia thus makes it necessary to reconsider the possibility that a long-lasting post-synaptic change may be contributing to the PTP found for the polysynaptic reflex in spinal cord, and perhaps in other situations.

PTP of the purely presynaptic variety, as in the spinal cord's monosynaptic reflex pathway, which shows only homosynaptic and not any heterosynaptic PTP, has been found to be highly frequency dependent and to require conditioning trains at much higher frequencies (Lloyd, 1949; Curtis & Eccles, 1960). The generation of slow EPSP, on the other hand, is relatively insensitive to the frequency of preganglionic impulses (Eccles & Libet, 1961). It seems likely that the unique ability of sympathetic ganglia (Eccles, 1957) to exhibit long-lasting potentiation following a low-frequency train or even after a single conditioning volley (Larrabee & Bronk, 1947) depends to an important degree on the generation of slow EPSP, although a contribution from presynaptic PTP appears to be likely. It should be noted that the 'natural' frequencies of discharge are very low in preganglionic fibres, when these are participating in the resting and reflex actions of the autonomic system (Bronk, Ferguson, Margaria & Solandt, 1936; Pitts, Larrabee & Bronk, 1941; Folkow, 1952; Douglas & Ritchie, 1957).

A relatively large total EPSP is required in ganglion cells to elicit postganglionic discharge because the firing level is that of the high-threshold somatic region (Eccles, 1963), rather than that of a low-threshold axon hillock region, as occurs in motoneurones (Eccles, 1957). One function of the slow EPSP in natural autonomic activity may be, then, to provide a kind of low-gain amplification. This may supply a margin which can raise many ganglion cells to their firing level, especially in the face of the depressant after-hyperpolarizations.

From the lack of any dramatic effects of small doses of atropine (0·5 mg/kg or less) on certain resting functions such as arterial blood pressure, one might be led to conclude that the slow EPSP is not a necessary factor in the ganglionic transmission of autonomic activities. A number of observations of atropine effects, however, do indicate that the slow EPSP mechanism plays an important role in at least certain autonomic functions; the possibility that it does so in other autonomic functions merits exploration. For example, the postural hypotension and vasodilatation (Kalser, Frye & Gordon, 1954), and the fall in peripheral resistance (Berry, Thompson, Miller & McIntosh, 1958) which occurs when a parental dose of 2 mg of atropine is administered to normal human subjects, is best explained by assuming that a 'partial blockade' of sympathetic ganglia has been produced (Miller, Kalser, Frye & Gordon, 1954); such a blockade could only apply to the slow EPSP, not to the well-known initial EPSP. The observation that stimulation of the preganglionic nerve to the bladder elicits a contraction with an initial quick phase, which is blocked selectively by nicotine, and a long-lasting slower-developing 'tonic' phase, which is blocked selectively by 0·1 mg/kg of atropine (Henderson &

Roepke, 1934, 1935) is explainable in terms of parasympathetic ganglionic discharge having been initiated by the fast and slow EPSPs respectively.

As a final point bearing on potential significance, it should be emphasized that the slow EPSP is elicited in structures which do not have any known interneurones or recurrent axon collaterals (Ranson & Billingsley, 1918; Johnson, 1918; Eccles, 1935*a*). Thus, the lengthy duration of these responses cannot be ascribed to repetitive neural activation. This raises the possibility that similarly long-lasting post-synaptic responses, as opposed to responses requiring multiple temporal and spatial activation patterns, could be contributing to the slow potentials and physiological processes which are uniquely prominent in the brain.

Inhibition. It has not been possible with the present approach to establish clearly the existence of synaptic inhibition in sympathetic ganglia, even though the evidence which was obtained is in good accord with the postulate of an inhibitory response. Indirect effects associated with the administration of dibenamine and reserpine *in vivo* can probably produce changes in the same direction as those expected from any blocking actions on an inhibitory response. One cannot, therefore, as yet conclude that the actions of these drugs were due to the specific elimination of an inhibitory post-synaptic potential.

Control periods, especially those in which a low arterial pressure comparable to that produced by these drugs was maintained, could also exhibit increases in post-ganglionic spike heights and relative reductions in ganglionic after-positivity. It seems likely that the partial asphyxia or anoxia of the ganglion lasting for an hour or more may lead to the alterations observed. In the case of the monosynaptic spinal cord reflexes, Lloyd (1953) has reported both a rise in reflex discharge and a reduction in the after-positivity that follows discharge, and Brooks & Eccles (1947) a rise in the focal (synaptic) potentials during the early phase of asphyxia. It has also been shown that the after-hyperpolarization following activity in autonomic nerve fibres is considerably more sensitive than is the action potential to metabolic disturbances such as anoxia (Greengard & Straub, 1962). Reinert (1963) has suggested that the increases in amplitude of the recorded post-ganglionic spike responses after administering reserpine or dibenamine, an observation reported earlier by Costa *et al.* (1961), are due to a progressive dehydration and shrinkage of the extracellular spaces during the long waiting period after giving the drug. This explanation seems improbable, at least for the present experiments: (*a*) fluids were administered intravenously (about 5–10 ml./hr) in all the *in vivo* experiments: (*b*) the reduction of the ganglionic after-positivity which often accompanied the rise in ganglionic and post-ganglionic spike response is in a direction contradictory to Reinert's suggestion.

The *in vitro* experiments were also inconclusive on this point: the concentration of dibenamine (1–2 μg/ml.) required for a complete blockade of the P synaptic potential in curarized ganglia often does depress the EPSP and the spike of the uncurarized, isolated ganglion to some extent; and the long wait required with reserpine introduces complications of slow but progressive deterioration of the preparation.

SUMMARY

1. A late surface-negative component is found to be superimposed on the positive after-potential recorded at sympathetic ganglia, following either single or repetitive orthodromic but not antidromic activation. It is

apparent in both isolated ganglia and those through which blood circulates *in situ*.

2. The slow-negative component appears to be identical with the late-negative (LN) synaptic potential which has been described in curarized ganglia. The time courses and amplitudes are similar, and both potentials are selectively eliminated by low concentrations of atropine.

3. The LN component appears to be generated in the ganglion cells which discharge the post-ganglionic impulses.

4. In view of these attributes of the LN potential and of its excitatory influence on the ganglion cell (see below), it seems advisable to refer to it as a slow EPSP (excitatory post-synaptic potential).

5. The recruitment or even maintenance of post-ganglionic discharge at low frequencies depends, at least in part, on the generation of the slow EPSP.

6. A major portion of the post-tetanic potentiation (PTP) demonstrable in the rabbit's superior cervical ganglion, by homosynaptic testing following the conditioning trains employed here, can be eliminated by a low concentration of atropine. A smaller fraction of such PTP was eliminated by atropine in the cat's stellate ganglion.

7. A considerable amount of PTP could be demonstrated even with heterosynaptic testing in the stellate ganglion; such PTP was virtually entirely eliminated by atropine.

8. It is concluded that two types of PTP can exist in sympathetic ganglia: (*a*) the well-known presynaptic type, which is atropine-resistant: (*b*) one due to the slow EPSP, which is atropine-sensitive and relatively independent of the frequency of the conditioning impulses.

9. Possible roles are suggested for the slow EPSP in the maintenance and transmission of physiological activities in the autonomic nervous system. The results also provide a clear demonstration that atropine can specifically block a physiologically significant process in sympathetic ganglia.

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