Unclassified (Security Classification)

Contractor: <u>Regents of the University of California</u>, <u>Berkeley 4</u> Contract No.: <u>DA18-106-AMC-161(A)</u>: <u>CP3-19975</u>

Final REPORT

Covering the Period

July 1, 1963 - June 30, 1964

Title: Synaptic transmission in sympathetic ganglia

ŧ

AD6099563

Frepared By

DDC RARME Benjamin Libet AM 14 VLIJIV DDC-IRA B January 15, 1965 Date:

Copy _ 24 of 24 copies

Unclassified (Security Classification)

n the state 2617

AD Accession No. Unclassified	D Accession No. Unclassified
Regents of the University of California Barbalay California Barbalay Califol 1 Synannic transmission	kegents of the Universisity of
Sympathetic ganglig - 1	Sympathetic Zanglia- Sympathetic Zanglia- Pharmacology autonomic
SIMPATHETIC IMANOMISSION IN PARTMACOLOGY AUTONOMIC : SYMPATHETIC GANGLIA - B. Libet nervous system	MERIALIU GAMELIA - D. LIDEL Remort No.2 Jan 1 64 14nr-8 illus nervous system.
Report No.2, Jan.1, 64 14 pp-8 illus	Contract DA 18-108-AMC-161 (A): 2. Contract DA 18-108-
Contract DA 18-108-AMC-161 (A): 2. Contract DA 18-108- CP3-19975 AMC-161 (A): CPS-19975	CP3-19975 AMC-161(A):CP3-19975
	Properties of the atropine-sensitive slow synaptic res-
roperties of the atropine-sensitive slow synaptic res-	ponses in memailai sympacticul bandina "c'o intere and y sed as to their 1) postsynaptic origin, 2) transmitter
sed as to their 1) postsynaptic origin, 2) transmitter	substances, and 3) durations of synaptic delays for each.
suoscances, and J) durations of synapric delays for each.	LUTTACEILUIAT STUDIES OU SINGLE SANGITON CELLS OF 105 9 1 Sympathetic ganglia are also reported in relation to
sympathetic ganglia are also reported in relation to 1) atropine-sensitivity of true after-porentials and	 atropine-sensitivity of true after-potentials and are- and post-synaptic blocking action of curariform
2) pre- and post-synaptic blocking action of curariferm	substances. The actions of other nicotinic blocking
<pre>% substances. The actions of other nicotinic blocking agents</pre>	agents (hexamethonium,mecumylamint) on frog ganglionic
responses are also reported. Developments in the technique	synaptic responses are and reported. Developments in the technique for intracellular recording in mammalian
for intracellular recording in mammalian ganglia are	ganglia are described. Unclassified
AD Accession No. Unclassified Regents of the University of 1 curved and a contract of the University o	AD ACCESSION NO. Unclassified Regents of the University of
California, Berkeley, Calif l Sympathetic ganglia-	California, Berkeley, Calif. 1 1. Synaptic transmission-
SYNAPTIC TRANSMISSION IN SYMPATHFTIC CANCIIL - R 14b+	SYNAFTIC TRANSMISSION IN SYMPatuctic Sangita - S SYMPATHETIC GANGLIA - B. Libet Pharmacology autonomic
Report No.2. Jan. 1.64 14 pp- 8. illus	Report No.2, Jan.1,64 14 pp-8 illus nervous system.
Contract DA 18-108-AMC-161 (A): 2. Contract DA 18-108-	Contract DA 18-108-AMC-161 (A): 2. Contract DA 18-108- CP3-19975 AMC-161 (A): 2. AMC-161(A):CP3-19975
roperties of the atropine-sensitive slow synaptic responses i in mammalian sympathetic ganglia were further analysed as	Properties of the atropine-sensitive slow synaptic res- ponses in mammalian sympathetic ganglia were further analy-
to their 1) postsynaptic origin, 2) transmitter substances, and 3) durations of summatic datasets	sed as to their 1) postsynaptic origin, 2) transmitter
lar studies on single gangion cells of frog's sympathetic	Intracellular studies on single ganglion ceils of frog's
ganglia are also reported in relation to 1) atropine-sensi- tivity of true after-potentials and 2) pre- and postsymantic	sympathetic ganglia are also reported in relation to 1) atropine-sensitivity of true after-potentials and
blocking action of curariform substances. The actions of other nicotinic blocking actions of	2) pre- and post-synaptic blocking action of curariform
on frog 8anglionic synaptic responses are also reported.	substances. The actions of other ricotinic blocking agents (hexamethonium, mecamylamine) on frog ganglionic
Developments in the technique for intracellular recording is in mammalian ganglia are described.	synaptic responses are also reported. Developments in the rechnique for intracellular recording in mammalian
	ganglia are described. Unclassified

AIMS AND PURPOSES OF WORK ON THE CONTRACT

Introduction. Two ganglionic potentials with much slower characteristics than those of the already known excitatory-post-synaptic-potential (EPSP) were discovered in curarized sympathetic ganglia in response to preganglionic volleys of impulses. In such ganglia, the EPSP is followed by longer lasting surface-positive potential (P) and a still longer late-negative (LN) potential (Laporte and Lorente de No, 1950; Eccles, 1952). The slow ganglionic potentials were found to be selectively depressed by low concentrations of stropine, which have no effect on the BPSP (Eccles and Libet, 1961). In addition, anti-adrenergic agents like dibenamine selectively abolished the P potential and depressed, to a lesser extent, the LN potential. On the basis of this and other evidence, a theoretical schema for the origin of these potentials was proposed (Eccles and Libet, 1961). In this, it was postulated that the P potential is an inhibitory hyperpolarizing response of the ganglion cells, mediated by an adrenaline-like transmitter substance; the latter would be released from chromaffine cells in the ganglion when these cells are cholinergically excited by preganglionic nerve endings in them. The LN potential was postulated to arise by action of acetylcholine (ACh) on muscarinic receptor sites on the ganglion cells; these would be in contrast to the nicotinic receptor sites on the same cells which mediate the EPSP response to ACh.

Subsequently, it was shown that the LN potential could be clearly demonstrated in normal, uncurarized ganglia (Libet, 1964). Since this LN synaptic potential was found to be associated with a correspondingly long period of facilitation, this response was named the "slow EPSP", in contrast to the well-known initial EPSP. The slow EPSP builds up greatly with repetition of preganglionic volleys, and then outlasts the end of a preganglionic train by tens of seconds. It provides therefore a form of post-tetanic-potentiation (PTP) (Libet, 1964) which is different from the PTP which has been ascribed to changes in the presynaptic terminals, as described earlier by Larrabee and Bronk (1947) and others. The slow EPSP, however, builds up to maximal levels even with the low frequencies of preganglionic impulses that are characteristic of the natural activities of the autonomic nervous system; this is in contrast to the high frequencies required for full development of presynaptic PTP.

In view of these properties of the slow EPSP, and of the probability that the P potential represents a long lasting inhibitory postsynaptic response in sympathetic ganglion cells, a further analysis of the nature and significance of these slow responses could lead to an important expansion of our knowledge of the physiological operations and pharmacological sensitivities of the autonomic nervous system, and perhaps of certain slow processes in the central nervous system.

Specific Aims.

a. The physiological nature of the slow synaptic responses were to be further investigated. This included attempts to obtain more definitive evidence of their postsynaptic origin, of their dependence upon presynaptic release of transmitter substance, and to establish quantitatively the synaptic delays involved in their onset, as well as their durations. b. Further evidence was to be sought bearing on the hypothesis that a catecholamine is involved as a transmitter in mediating the P potential. This has centered chiefly on the testing of the effects of such compounds on the level of the transmembrane potential difference of ganglion cells.

c. Further testing of the sensitivities of the slow synaptic responses to pharmacological agents of the ganglion-blocking type was to be carried out.

METHODS

For experiments in which recordings were made from the surface of the ganglion, the isolated preparation was mounted in a chamber, as described elsewhere in detail (Eccles and Libet, 1961; Eccles, 1952). The mammalian ganglion utilized was commonly the superior cervical of the rabbit, but others, such as the cat's coeliac or stellate, were also employed. The sheath was removed from ganglion and post ganglionic nerve, to reduce external shunting and increase the penetration of chemical agents. In the frog, the sympathetic chain was utilized, with a portion of the sciatic bundle containing the postganglionic bundles of the 9th or 10th ganglion in the chain. Ganglia were mounted on wire electrodes, Pt for stimulation of the preganglionic fibers and Ag-AgCl for recording at the ganglion and postganglionic sites. The preparation was bathed in a Ringer-Krebs solution, which for mammalian ganglia, was maintained at 37-38°C and bubbled with 95% 02 -5% CO_2 . In recording, the preparation and electrodes could be brought into air out of the saline by tilting the chamber. Responses were recorded, with DC amplification throughout, between the active lead and an indifferent one generally located at the crushed end of the postganglionic nerve.

Intracellular recordings from single ganglion cells were made with glass micropipettes having tip diameters of less than 0.5 microns. Successful experiments of this kind were confined to the frog's sympathetic ganglia, which can be penetrated by the electrode, though not without some difficulty. The impenetrability of mammalian ganglia, owing to an all-pervasive tough connective tissue structure throughout its interior, requires special development of techniques to drive the microelectrode into the ganglion. The experiences with this will be described in the section below, on Experiments etc. The pre-and postganglionic trunks of the frog ganglia were drawn into glass tubes, filled with saline, which served as stimulating or recording leads at these points, according to the method of Furshpan and Potter (1959). The microelectrode was advanced by an oil-filled drive operated from another table. The Ag-AgCl wire in the micropipette fed into the usual cathode-follower impedence-converter, and from there into DC amplifiers or meters.

EXPERIMENTS, TESTS AND RESULTS

Synaptic potentials, uncontaminated by any propagated action potentials and their associated after-potentials, may be recorded at the surface of curarized sympathetic ganglia. A single preganglionic volley then may elicit a series of three ganglionic potentials (Fig. 2; see also Eccles, 1952), representing the initial EPSP, a surface-positive potential (P), and a late negative one which has the properties of a slow EPSP (Libet, 1964).

<u>Decremental propagation in postganglionic nerve</u>. With an electrode positioned at different points along the postganglionic nerve (see diagram, Fig. 1), the potentials recorded at the curarized mammalian ganglion may be seen to fall off rapidly with increase in distance from the end of the ganglion (Fig. 2, A-F). For such an experiment all postganglionic nerve bundles except one were cut off, and the remaining one was exceptionally well cleaned of adhering sheath tissue; presumably the decrement with distance would be somewhat less steep under natural conditions with a lower extracellular resistance. The change in the first 0.5 mm of the nerve bundle may not be very significant as it is impossible to avoid trapping small amounts of the saline solution at the junction of the ganglion with the postganglionic bundle, when the preparation is raised into air for recording.

All three ganglionic responses, the initial EPSP, the P potential, and the slow EPSP, fall off with increasing distance from the ganglion, and do so at approximately equal rates. It is difficult to precisely estimate the rates of such decrementation from the available data, and still more so the times for achieving the maximum amplitude of each potential at each point on the postganglionic nerve. The times to the true maxima of the two slow responses are in any case obscured by overlapping with the preceding synaptic responses. The rough approximations obtainable for the space and time constants, however, are in keeping with the orders of magnitude expected for passive cable conduction in C fibers.

<u>Preganglionic</u> nerve recordings (see diagram, Fig. 1), on the other hand, show no evidence of these ganglionic potentials, even when the proximal electrode is placed less than 1 mm away from the beginning of the ganglion (Fig. 2, H). Only the diphasic recordings of the action potentials in the preganglionic nerve fibers are detected in the superior cervical preparations, during the application of trains of stimuli at up to 40 per sec for 1 sec or less.

The slow synaptic potentials are thus seen to be localized to the region of the ganglion cell bodies and to decrement rapidly in the postganglionic axon, in the same way as the initial EPSP.

<u>Effects of lowered Ca++/Mg+ ratios</u>. The dependence of the slow potentials on presynaptic release of ACh had already been indicated by the effect of botulinum toxin (Eccles and Libet, 1961); this was now further examin^d by subjecting the ganglia to external solutions with low Ca/Mg ratios. It has been shown that such treatment selectively depresses presynsptic release of transmitter substances in response to presynaptic impulses (del Castillo and Katz, 1954; Hutter and Kostial, 1954).

Suitable lowering of the normal ratio of the external concentrations of Ca/Mg depressed or abolished all postsynaptic responses. In the curarized mammalian ganglion all three postsynaptic potentials were affected similarly; if anything, the P potential and slow EPSP were depressed more readily than the initial EPSP. In the uncurarized ganglion, when postsynaptic discharge of impulses was abolished by this treatment, no postsynaptic potentials of any kind remained. These effects were initially observed by simply raising the external MgCl2 concentration from lmM/1 to 10mM/1; subsequently raising the MgCl2 concentrations as high as 20 mM/1 to block conduction in some axons (Katz and Miledi, 1964) Ca/Mg ratio was also lowe ed by lowering Ca and raising only moderately the Mg concentration. With all CaCl2 omitted and MgCl2 raised to only 5 mM/1 in the Krebs-Ringer solution, all postsynaptic responses were abolished; monitoring the preganglionic nerve in this case showed that the conduction of preganglionic impulses was not significantly affected under these conditions. These results demonstrate that the generation of the P potential and the slow EPSP are at least as sensitive as the initial EFSP to a lowering of the external Ca/Mg ratio.

Latency of the postsynaptic P potential in mammalian ganglia. In order to display the onset of the P potential without contamination by the initial EPSP, the latter was completely suppressed by strong curarization. To accomplish this a concentration of d-tubocurarine (d-TC) is required which is about 10 times that needed to just block the generation of a postganglionic propagated spike by a single orthodromic volley. At these high concentrations of curarizing agent (about 125-150 µg/ml) the slow postsynaptic potentials, especially with single orthodromic volleys, are also depressed, but are still definite enough for the purpose of studying latency (Fig. 2, C). Incidentally, the presence of P and slow EPSP responses in the absence of any initial EPSP provides some of the evidence for regarding them as independent processes, rather than as some sort of after-effects of the EPSP (see Eccles and Libet, 1961).

That the latent period before onset of P potential is a considerable one is evident even in the slow sweep record of Fig. 30. When the sweep speed is increased to facilitate more precise measurements of latency, however, it becomes difficult to pinpoint the onset of P with such small amplitudes of the potential (Fig. 3D and F). Even so, the minimum latency appeared to be about 40 msec. This value was confirmed when the onset of the response was made much more distinct by post-tetanic-potentiation (PTP) of the postsynaptic P potential. PTP of the P potential could be accomplished without any disturbing increase in the amplitude of the initial EPSP, in the strongly curarized gauglion. Fig. 3 - G, H, and I show the responses to single preganglionic volleys delivered at increasing times after the end of a 15 sec train of preganglionic volleys at 60/sec. The strength of stimulus in this series was sufficient to excite all the B and C fibers in the preganglionic nerve; this is much more optimal for eliciting P response than is stimulation of B fibers alone, as in Fig. 3 -D, E (see Eccles and Libet, 1961).

The long latent period of 40 msec for the postsynaptic P potential is to be compared to the short one of 5-6 msec for the onset of the first (Sa) discharge in the uncurarized ganglion (Fig. $3-A_2$). The latent period for the onset of the initial EPSP as measured in a lightly curarized ganglion, is approximately the same as that of this Sa discharge. These latency values of course all include conduction times from the point of preganglionic stimulation to the presynaptic terminals. The latent period of the second, or S5 portion of the postsynaptic discharge (Pig. 3A), (also Fig. 4A) was about 15 msec longer than that for the Sa portion; this difference can be accounted for by the glower conduction velocity of the preganglionic C fiber group as opposed to the B fiber group (Feeles and Libet, 1961). The onset of the P potential is, however, initiated by the faster B impulses, whether these are delivered alone (Fig. 31 E) or are followed by the C impulses (Fig. 3F - I). Subtracting the 5-6 msec latency of the Sa postsynaptic discharge thus leaves us with a net additional delay of about 35 msec for the onset of the postsynaptic P potential, over and above that required for the onset of the initial BPSP.

Latency of the slow EPSP in mammalian ganglia. To demonstrate clearly the Veronset of the late negative postsynaptic potential, the slow EPSP, possible interference or masking by the P potential as well as the initial EPSP had to be eliminated. This could be accomplished in the strongly curarized ganglion (Fig. 4C) by the addition of a suitable concentration of dibenamine (Fig. 4D-G). The latter drug can block the P response completely while depressing the slow EPSP only

ed

n

4

partially (Eccles and Libet, 1961). It can then be seen clearly (Fig. 4C -G) that the onset of the slow EPSP has a latent period in the range of 200-300 msec in different preparations. Some ganglia exhibit little or no postsynaptic P potential in response to single preganglionic volley. In such preparations the onset of the slow EPSP may be seen distinctly even in the lightly curarized condition and with no dibenamine added. These circumstances apply to the recordings shown in Fig. 4 from a celiac ganglion of a cat. When the initial EPSP in I-A of Fig. 5 is recorded at a slower sweep speed it can be seen that only sometime after the return of the ganglionic potential to the resting base line does the slow EPSP begin to appear (arrow, Fig. 5, I-B). The onset of the slow EPSP is delayed by somewhat more than 300 msec from that of the initial EFSP in this case. Column II in Fig. 5 shows the comparable responses after the addition of atropine (0,1 μ g/ml), which can selectively block the slow EPSP (Eccles and Libet, 1961). At still slower sweep speeds (Fig. 5, I - C, D.) the more complete course of the slow EPSP is visible.

Slow postsynaptic potentials in frog ganglia. Concentrations of d-TC of 5 to 10 μ g/ml were found to block postganglicnic discharge in response to preganglionic volleys, leaving an EPSP of appreciable size instead. Following short trains (0.25 - 1 sec duration) of repetitive preganglionic volleys, at 10-80 per sec, a distinct P potential could appear, when the tetanically summated EPSP's suddenly collapse at the end of the train (Fig. 6). This P potential has a duration of several seconds and maximum amplitudes equal to about 10% of the uncurarized spike amplitude.

The P synaptic potential was elicited only when the stimulating electrode on the thoracic chain was located less than 10 mm anterior to the ganglion (no. 9 or 10 in the chain), as seen in Fig. 6. This means that only preganglionic fibers entering the chain within one or two segments above the responding ganglion could elicit this slow synaptic potential. Stimulation of the thoracic chain more anteriorly could still elicit a large EPSP, or large spike discharge in the uncurarized ganglion, but could not generate any P potential. The preganglionic fibers which elicit the P synaptic potential, then, can be different and separate from those which elicit only an EPSP.

Late negative synaptic potentials (slow EPSP) were ordinarily not seen in the frog's sympathetic ganglia (except in some instances after higher frequency trains (60-80/sec) of preganglionic volleys). This is in contrast to the large ones observable in mammalian ganglia (Eccles 1952) and the rather small ones reported for turtle ganglia (Laporte and Lorente de N5, 1950).

The P potential of frog ganglia could be blocked by low concentrations of <u>atropine</u> $(0.1 - 0.2 \ \mu\text{g/ml})$ as in mammalian ganglia. Dibenamine, however, had only a moderate depressing action, in concentrations $(3 \ \mu\text{g/ml})$ which abolish P completely in mammalian ganglia. The different responsiveness of the frog to certain autonomic blocking agents has already been documented by Burnstock.

Intracellular recordings in single cells of frog ganglin.

a) Uncurarized ganglia. The general form and properties of the discharge of a frog ganglion cell, ortho-and antidromically, as already described by Nishi and Koketsu (1960) and Blackman, et al. (1963s), were confirmed by us. Ample incidence of spontaneous miniature postsynaptic potentials was also seen, as reported by Blackman, et al (1963b). The hyperpolarizing after-potential which follows discharge of a ganglion cell which has been fired antidromically via its own axon, is a "true" after-potential; it should not contain any postsynaptic component such as the P potential (which is also presumably a hyperpolarizing action). Atropine (0.1 μ g/ml) was found to have no effect on this true after-hyperpolarizing potential, in antidromic responses. Since such concentrations of atropine completely suppress the P synaptic potential, as generated orthodromically in curarized ganglia, this proves that the P potential involves a different mode of production than the true-hyperpolarization; that is, it supports the contention that the P potential is a postsynaptic potential elicited by synaptic transmitter substances, one of which can be antagonized by atropine.

b) <u>Curarized ganglia</u>. To demonstrate a postsynaptic hyperpolarising response to preganglionic impulses, as distinguished from a true after-potential of this kind, it is necessary to block the firing of impulses (action potentials) by the ganglion cell. This can be done by applying a curarizing agent in a concentration sufficient to depress the EPSP below the cell's firing level. The P synaptic potential, recordable at the surface of a ganglion, survives this kind of treatment (Eccles, 1952: Eccles and Libet, 1961).

With a microelectrode in place inside a ganglion cell, the application of either d-TC, or dihydro- β -erythroidine, in concentrations just adequate for blocking postsynaptic firing (about 5 μ g/ml for either one) appeared to depress not only the postsynaptic response but also to depress and block transmission in the presynaptic terminals as well. The evidence for the letter was that the EPSP either disappeared suddenly (in an all-or-none fashion) shortly after the cell spike had been blocked (Fig. 7C-E), or no EPSP response could be elicited at all after the cell spike was lost. In addition, during the short time when an BPSP was still elicited after loss of the spike, the latent period of its onset increased distinctly over what it was before applying the curarizing agent (Fig.7A-B). Similarly, when the weakest blocking concentration of these drugs was applied to a ganglion before the impalement of a ganglion cell by the microelectrode, subsequent penetration of cells revealed the following. Either the cell could still respond to an orthodromic volley with a spike discharge, although at times somewhat delayed by a reduction in the rate of rise of EPSP, or no response (cell spike, or EPSP) was present at all. In the latter case, the cell could still respond to an antidromic volley with a normal discharge.

Depression of presynaptic transmission by TEA has also been reported by Riker (1963), with microelectrode studies of frog ganglia, although no mention of it was made by Blackman, et al (1963). The question arises as to why it is possible to record an EPSP from the surface of the ganglion with the use of these drugs. It may be that there is some slight mechanical injury or distortion of the presynaptic terminations when a micropipette is being pushed through the ganglionic mass and into a cell. This may increase the susceptibility of these terminals to a blocking action by curarizing agents; such an increase in suceptibility by mechanical factors has been observed for the neuromuscular junction (Katz and Miledi, unpublished).

Blockade of conduction in presynaptic terminals, regardless of the special causes, obviously would abolish all postsynaptic responses. Consequently, a slow postsynaptic hyperpolarizing response will have to be looked for under conditions which more selectively block the EPSP alone; this aspect of the study could thus not be completed at present. Testing of other nicotinic blocking agents, which

6

might not possess the same tendency to depress presynaptic terminals as well, has however, been carried forward (see below).

c). Effect of epinephrine. With a ganglion cell impaled by a microelectrode, the effect of adding epinephrine on the resting transmembrane potential difference was observed. In order to achieve a rapid entry of the substance into the vicinity of the cell, a tiny volume (0.03 ml) of solution was delivered into the bath from the 10 μ tip of a glass pipette. The latter had been previously fixed in position with its tip a few mm away from the ganglion, and the solution was expressed slowly (over a 10 sec period) from the pipette by a remote micro-syringe which was connected to the pipette via a long polyethylene tube. Solutions in the pipette contained 1 mg/ml of epinephrine or more epinephrine in frog Ringer's.

Within 15 seconds after extrusion of the epinephrine solution, there occurred a distinct slow rise of several millivolts in the resting membrane potential, which was then sustained. Similar extrusions of Ringer's solution alone gave no such change; the change expected to be seen if there were any motion of the microelectrode upon the extrusion of solution would be a reduction in resting potential, due to damage to the cell membrane. Such depolarizations are all too readily observed, if the solution is extruded too vigorously and in too large a volume. A hyperpolarizing action of epinephrine is in accord with its postulated role as a mediator of the P synaptic potential (Eccles & Libet, 1961) and with the inhibitory effect of injected epinephrine on postganglionic discharge in response to preganglionic volleys (Marazzi, 1939; Lundberg, 1952; Eccles and Libet, 1961).

Additional pharmacological agents for selective blockade of EPSP. Since d-TC and dihydro- β -erythroidine both are prone to produce presynaptic block in frog ganglia it was desirable to test other blockers of nicotinic receptors which are chemically rather different from the curare group. Hexamethonium is one such compound, which is an excellent blocking agent in mammalian ganglia. In frog ganglia, however, hexamethonium in concentrations up to 150 µg/ml hardly depressed the synaptic response. Blackman, et al. (1963) did report blockade by still higher concentrations of this drug (about 225 µg/ml, i.e. $7x10^{-4}$ M, but effects that require such high dosage did not seem worth pursuing.

Mecanylamine, however, turned out to be a very effective blocking agent in frog ganglia. As little as 2-4 μ g/ml could block orthodromically elicited post-ganglionic discharge and depress the EPSP. Under these conditions a good P potential could be seen to follow a short preganglionic train of impulses (Fig. 6). There was not time, before the termination date of this contract, to utilize this drug with intracellular recording technique.

<u>Development of intracellular technique with mamialian ganglia</u>. Mammalian ganglia must of course be maintained at 37-38°C and with suitable oxygenation and stirring of the solution. A special chamber and warm-water bath was constructed to provide this while at the same time supplying baffles against the sudden motions of the fluid from the 02 bubbling. Photographs of this chamber are shown in Fig. 8. Transmitted illumination of the ganglion is provided by reflection from a mirror mounted in the bath below the ganglion compartment. The stumbling block to successful intracellular recording was not the insbility of the microelectrode to penetrate the dense connective tissue meshwork which pervades the interior of such ganglia. Ganglion cells at the very surface of a desheathed ganglion can occasionally be impaled (as reported by Eccles, 1955, 1963), but these are not suitable for the study of the slow synaptic responses; it should be recalled that the latter are postulated to involve diffusion of transmitter substances through intercellular spaces.

Preliminary attempts were made to facilitate penetration by treating the ganglion with preparations of proteolytic enzymes (trypsin), or with enzymes to hydrolyse other portions of the interstitial stroms (collagenase, elastase, hyaluronidase). After action by an enzyme was sufficient to permit penetration by the microelectrode, however, there was also a considerable loss in the postganglionic response to pregenglionic volleys. Perhaps a more thorough investigation will uncover conditions in which a differential between the effects on penetrability and on synaptic transmission can be achieved. As far as tested here, such enzymatic methods appeared to be unsuitable.

Penetration into certain other tissues (e.g. retina) has been improved by some investigators by vertically vibrating either the whole preparation against the microelectrode, or vibrating the microelectrode as it is lowered into the tissue. We have now constructed a light-weight holder for the electrode which includes a small slectromagnetic coil for vertical vibration of the electrode (Fig. 8). Freliminary tests have indicated that this device may produce satisfactorily penetration, but further work on the optimum parameters of excursion, frequency, and pulse duration of the vibratory oscillations is still necessary.

SUMMARY OF RESULTS

Properties of the atropine-sensitive, slow synaptic responses in mammalian sympathetic ganglia were further analysed. It was shown that both the surfacepositive (presumably hyperpolarizing) potential (P), and the late surface-negative potential ("slow excitatory postsynaptic potential," or slow EPSP) are localized to soma-dendritic region, just as is the well-known initial EPSP (which is curaresensitive). All these postsynaptic responses were abolished by lowering the Ca/Mg ratio in the external medium; i.e. they all depend upon release of some transmitter substances at presynaptic terminals. The slow responses were found to have extraordinarily long latent periods for their onset (about 35 maec for the P potential, and 200-300 maec for the slow EPSP, exclusive or conduction time to the ganglion).

A slow P postsynaptic potential could also be demonstrated in the curarized sympathetic ganglis of the frog, if preganglionic fibers from spinel segments suitably close to the ganglion under study were stimulated. This P potential was also easily blocked by stropine, but only partially by dibenamine.

Intracellular recordings from single ganglion cells of the frog were made. The true after-potentials which follow antidromic discharge of such cells are not affected by doses of atropine which depress the P synaptic response. The curarizing agents, d-tubocurarine and dihydro- β -erythroidine, not only depress the EPSP but also appear to block conduction in presynaptic terminals of such preparations. Clear evidence and analysis of the curare-insensitive slow post-synaptic responses at the single cell level will, therefore, have to await the use of more suitable blocking agents. A catecholamine, epinephrine, was found to produce a hyperpolarization of the membrane of ganglion cells in the frog. This is in accord with its postulated role in the generation of the P synaptic potential.

Additional micotinic blocking agents were tested on the frog's sympathetic ganglia. Hexamethonium required concentrations above 150 μ g/ml to produce appreciable depression of the ETSP, and is therefore unsuitable. Mecamylamine, 2-4 μ g/ml, did depress the ETSP strongly, without blocking the slow P potential.

Developments of technique for intracellular recording in mammalian sympathetic ganglia were described. A device for vibratory oscillation of the microelectrode appears to improve the penetratics of the electrode into the ganglion.

REFERENCES

1.	Blackman, J. G., Ginsborg, B. L., and Ray, C. (1963a). J. Physiol. 167, 355-373.
2.	Blackman, J. G., Ginsborg, B. L. and Ray, C. (1963b). <u>J. Physiol. 167</u> , 389-401.
3.	de Castille, J. and Katz, B. (1954). <u>J. Physiol</u> . <u>124</u> , 560-573.
4.	Eccles, B. M. (1952). <u>J. Physiol. 117</u> , 181-195.
5.	Eccles, R. M. (1955). <u>J. Physiol.</u> <u>130</u> , 572-58
6,	Eccles, R. M. (1963). <u>J. Physiol</u> . <u>165</u> , 387-391.
7.	Eccles, R. M. and Libet, B. (1961). J. Physiol. 157, 484-503.
8.	Furshpan, Z. J. and Potter, D. D. (1959). <u>J. Physiol</u> . <u>145</u> , 289-325.
9.	hutter, O. F. and Kostial, K. (1954). J. Physiol. 124, 234-241.
10.	Katz, B. and Miledi, R. (1964). <u>J. Physiol</u> . <u>171</u> , 10-11P.
11.	Laporte, Y. and Lorente de No, R. (1950). <u>J. cell. comp. Fhysiol. 35</u> , Suppl. 2, 61-106.
12.	Lerabee, M. G. and Bronk, D. W. (1947). J. Neurophysicl. 10, 139-154.
13.	Libet, B. (1964). J. Physiol. <u>174</u> , 1-25.
14.	Lundberg, A. (1952). Acta physiol., scand. 26, 252-263.
15.	Marazzi, A. S. (1939). <u>J. Pharmacol</u> . <u>65</u> , 395-404.
16.	Nishi, S. and Koketou, K. (1960). J. cell. comp. Physiol. 55, 15-30.
17.	Riker, W. K. (1963). The Pharmacologist, 5, 242.

CONCLUSIONS AND RECOMMENDATIONS

This work has added to our understanding of the synaptic mechanisms in sympathetic ganglia and of the ways in which low doses of atropine may act in the body. It also supports the contention that there is an adrenergic (inhibitory) synaptic mechanism operating in ganglia, in addition to the well-known cholinergic one. It should be noted that the activation of this adrenergic mechanism by preganglionic impulses is also sensitive to blockade by atropine.

Atropine can affect brain function, and has been shown in recent years to block spacifically cholinergic effects at certain sites in the brain. It is recommended that analyses of the effect of cholinergic agents and atropine on brain function take into second the possibility that these effects are transpiring at postsynaptic sites which generate slow synaptic responses, of the types seen in sympathetic ganglia.

- Fig. 1 Diagram of electrode arrangements. Stimulating electrodes, S were on the preganglionic nerve (cervical sympathetic). Ganglionic potentials were recorded with electrodes marked G, those on postganglionic nerve with the Post-G electrodes, using a common indifferent on the crushed end of the nerve. The active Post-G electrode could be moved to different positions along the nerve. Potentials on the presynaptic side were recorded with the Pre-G electrodes, one placed at the junction of the preganglionic nerve with the ganglion, the other utilizing the usual ground electrode 8 mm away on the preganglionic nerve.
- Fig. 2 Slow synaptic potentials recorded along postganglionic and preganglionic nerve. Superior cervical ganglion, curarized with dihydro-β-erythroidine (25 µg/ml); response to single maximal preganglionic volley (B and C fibers) in each case. Top tracing, 1, of each pair, is potential at Post-G electrode in A-G, Pre-G electrodes in H; bottom tracing, 2, is simultaneous record of ganglionic potential at G electrode. The distance from the ganglion to the active lead on postganglionic nerve is given above each tracing, in A-G. Calibration and time shown in C and F apply to all in A-G, except for the faster sweep speed in G.
- Latency of P synaptic potential. Superior cervical ganglion, responses to single preganglionic volleys. Ganglionic potentials, except for Fig. 3 Post-G record in top tracing, 1, in A. Stimulus at maximal Sa spike (B fiber) strength in D and E, at max. Sa and Sb spike (C fiber) strength (as in A) in all others. A, before curarization; B, same as A-2, but at 5 fold gain and slower sweep, to show positive after-potential and smaller late negative (LN) potential (spikes are off the screen). d-tubocurarine-Cl, 125 µg/ml, added before C. (Note only P and LN synaptic potentials in C; initial EPSP completely suppressed). D, before PTP; E, 60 sec. after a 15 sec train of preganglionic volleys at 60 per sec. F, before PTP; G, H, and I taken at 30, 90, 240 sec. respectively after a 15 sec train at 60 per sec. Arrows in D-I indicate beginning of P potential (which is difficult to specify in D, as indicated by additional dotted line). Voltage calibration in F applies to C-I; time in F applies to D-I, C having same sweep time as in B. (The regular pips seen in D-I are distorted 60 cycle artifacts).
- Fig. 4 Latency of late negative response (slow EPSP). Superior cervical ganglion, responses to single preganglionic volleys (15V stimulus, maximal for B and C fibers, except for 6V stimulus for B fibers only, in D and F). All ganglionic potentials (except for Post-G in A-1). A, before curarization; B, same but higher gain and slower sweep (note LN response here also). d-tubocurarine-Cl added before C (125 μ g/ml; raised to 150 μ g/ml before D). Dibenamine (4 μ g/ml) added before D (note that this abolished the P responses, as well as the small initial EPSP which was still evident in C). Arrows indicate onset of LN potential (slow EPSP). Voltage calibration in C applies to C-G; time in F for B-C and F-G, time in E for D-E.
- Fig. 5 Latency of slow EPSP in lightly curarized ganglion. Coeliac ganglion of cat, curarized with dihydro- β -erythroidine, 17.5 μ g/ml (note the very small spike still present on some responses in a 20 per sec train, as seen in E). A-D, ganglionic responses to single preganglionic volleys, at increasingly slower sweeps to demonstrate the LN component. Arrows in I-B and I-C indicate onset of slow EPSP. Column II tak γ after adding atropine, 0.1 μ g/ml, which selectively abolishes the slow EPSP. Voltage calibration as in A throughout; sweep speed as indicated (50 msec per similar div., in E).

Legends for Figures (continued)

- Fig. 6 <u>Slow synaptic potentials in frog ganglis</u>. Last ganglion of sympathetic chain of bullfrog (R. catesbiana). Ganglionic responses to a 0.5 sec train of maximal preganglionic volleys at 40 per sec. A, stimulus electrode on sympathetic chain several segments (16 mm) cranial to the recorded ganglion; in B-E, stimulus applied at only one or two segments (6 mm) cranial. A-C, after addition of mecanylamine, 4.5 µg/ml, for nicctinic blockade. (Note P potential following the stimulus period in B, but not A). Dibenamine, 3 µg/ml, added before C (note moderate depression of P after and evidently during the stimulus train). D and E, another preparation, after addition of d-tubocurarine-Cl, 22.5 µg/ml, with the further addition of atropine sulfate, 0.1 µg/ml, before E. (Note P potential in D is largely gone in E). Voltage and time calibrations same throughout.
- Fig. 7 Intracellular recordings of frog ganglion cells: effects of curarization. Responses to single orthodromic volleys (A-E) and to antidromic (F-G). A and F, before curarization, B-E and G after addition of dihydro-βerythroidine to the end of the bath distant from the ganglion (final conc. 20 µg/ml). (Note that initial effects of the drug were to slow the rise of the EPSP and also its latency for onset, B vs A; no effects are observed on antidromic responses, G vs F). C, taken 3 min after B; D, 0.5 min after C; E, 2.5 min after D. (Note that the EPSP decreased in amplitude to zero rapidly; reduction to zero amplitude occurred even with final concentrations only a few per cent of that here, if the whole chamber content was changed to include the drug before impaling the cell). Voltage scale same throughout; time equal 10 msec per div. in A-B and F-G; 50 msec in C, D, E.
- Fig. 8 <u>Chamber and electrode vibrator for intracellular recording in mammalian</u> <u>sympathetic ganglion</u>. Photographs of the bath, chamber and electrode arrangements. Oxygen is bubbled into one compartment behind a baffle. Ringer's is introduced continuously at one end of the ganglion compartment and sucked off at the other end, to maintain a constant level. The preganglionic trunk enters a separate oil-filled compartment through a small hole, so that it can be stimulated electrically without excessive artifact.



A

D

Figure 1

.

ż





.

.

. -





Figure 4

• •

ł





.

