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LIBERATION OF ACETYLCHOLINE BY THE PERFUSED SUPERIOR CERVICAL GANGLION

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IN earlier experiments from this laboratory on the perfused ganglion preparation, stimulation of the preganglionic nerve regularly caused the appearance of acetylcholine (ACh.) in the venous effluent [Feldberg & Gaddum, 1934], while stimulation of the vagus, of which the nodose ganglion was included in the perfusion, or of the sympathetic post-ganglionic branches, had no such effect [Feldberg & Vartiainen, 1934]. This and other evidence led to the definite suggestion that acetylcholine is liberated at the synapses by the arrival of preganglionic impulses, and plays an essential part in the synaptic transmission of the excitatory process, a view which much later evidence has further supported. Recently Lorente de Nó [1938], with the object of testing other possibilities as to the site of liberation of acetylcholine in the ganglion, has repeated the earlier experiments, using essentially the same methods, with modifications of detail. In a proportion of these experiments, especially when the methods used by Feldberg *et al.* were closely followed, Lorente de Nó obtained similar results. In other experiments his results were irregular, and, being impressed by the possibility of damage to the ganglion during its preparation and perfusion, he elaborated precautions to protect it from injury. These ultimately involved a very prolonged dissection in a moist room at 34° C., and perfusion, in some cases at least, at low pressures. Under these conditions, although the highest outputs of acetylcholine were still usually seen with preganglionic stimulation, it frequently appeared at other times, and sometimes failed to appear in response to preganglionic impulses. Lorente de Nó concluded that ACh. may be liberated in the ganglion when no impulses are arriving at the synapses, that its liberation is favoured by injury to the ganglion, and that its "metabolism is not a process which is specific to

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synaptic junctions". The experimental findings on which he based this conclusion may be detailed as follows:

(1) The liberation of significant amounts of ACh. in the absence of stimulation.

(2) Liberation of ACh. by stimulation of the postganglionic sympathetic fibres, or of the vagus, as well as by stimulation of preganglionic fibres.

(3) Delayed output of ACh., outlasting the stimulation by many minutes.

(4) Failure of preganglionic stimulation to discharge ACh. from ganglia which had been prepared with special precautions to avoid injury.

(5) Histological evidence of severe damage in those ganglia which had liberated ACh. on stimulation.

At the time of the publication of Lorente de Nó's paper I had for some time been engaged with experiments on the perfused ganglion, using an improved method of perfusion devised in co-operation with Dr G. L. Brown, in which the perfusion fluid was diluted blood instead of simple Locke's solution. Confirmation of the fundamental points of the earlier experiments had not been the direct object, but I had had the opportunity, in every experiment, of observing the close correspondence between the appearance of ACh. in the venous fluid, and the effective stimulation of the preganglionic nerve. With the improved perfusion method the ganglion was regularly maintained in good condition, without perceptible oedema, for several hours, during which repeated short periods of preganglionic stimulation had been observed to cause practically identical outputs of ACh. There was, accordingly, a favourable opportunity of repeating the original experiments under better conditions, and with special attention to the points at which Lorente de Nó had obtained results conflicting with those of the earlier workers. It will be seen that the results of my experiments are in close agreement with those of Feldberg & Gaddum and Feldberg & Vartiainen, and that I have failed to reproduce the irregularities observed by Lorente de Nó, even when I have varied the conditions with the object of favouring their appearance.

METHODS

Cats under chloralose anaesthesia were used in all the experiments. The dissection prior to perfusion was as described by previous workers [Feldberg & Gaddum, 1934; Feldberg & Vartiainen, 1934]. The vagus nerve and accompanying structures, with the exception of the

sympathetic postganglionic trunk, were tied between the ganglion and the skull, but not cut. During the dissection the wound was frequently bathed with warm saline, and great care was taken to avoid any mechanical injury to the ganglion. Elaborate precautions to prevent cooling and drying were not taken, and the temperature of the ganglion doubtless fell considerably during the operation, which usually required 30–40 min. for its completion. As soon as the dissection was finished, heparin was injected into the general circulation, in a dose sufficient to keep the blood incoagulable for the whole period of the experiment. The animal was then usually left, with the ganglion protected by closure of the wound and still retaining its normal blood supply, for an hour or more before the actual perfusion was begun.

In most of the experiments the perfusion fluid was a tenfold dilution of the cat's own heparinized blood in normal Locke's solution, with the addition of 1 : 250,000 eserine sulphate. The blood was diluted immediately it was drawn, and then passed twice through a No. 3 Jena sintered-glass filter. A small Dale-Schuster pump maintained a slightly pulsatile perfusion pressure of 100–150 mm. Hg; at these pressures the perfusion rate through the ganglion preparation was from 0·5 to 1·0 c.c. per min. A glass-wool filter was interposed between the pump and the arterial cannula. The perfusion fluid was warmed to a constant temperature of 38–39° C. by means of a coil of resistance wire built into the cannula, which was of the type devised by Gaddum and described by Feldberg & Vartiainen [1934], but shorter, and with the heating coil extending almost to the nozzle inserted into the artery, the fluid, containing blood, being thus at no point heated above 40° C.

The temperature in the neighbourhood of the ganglion was not lower than 35° C. during the course of the perfusion. In a few experiments, which will be separately considered, the fluid used was plain Locke's solution containing 1 : 250,000 eserine sulphate, perfused either by means of the pump, or by static pressure from a raised reservoir. The nerves to be stimulated were placed, at the beginning of the experiment, on glass-shielded platinum or silver electrodes, or in fluid electrodes of the Collison pattern, and left without disturbance until the experiment was completed. The stimuli were in all cases condenser discharges at a frequency of 10 per sec. A continuous record of the response of the nictitating membrane provided a check on the effectiveness of pre- or postganglionic stimulation.

The perfusate was collected in ice-cooled vessels, and either tested immediately on the leech preparation, or kept in ice until the assay could

be made. Control experiments showed that there was no appreciable destruction of ACh. by cat's blood at the dilution employed, and with the amount of eserine added, when kept at 0° C. during a period of several hours. The leech strips used always responded well to ACh. in 2×10^{-9} dilution, and usually to considerably lower concentrations.

Experimental

Failure of acetylcholine to appear in the perfusion fluid in the absence of stimulation

The presence of blood in the perfusion fluid, when this is first applied to the test preparation, sometimes provokes a small contraction, even when no ACh. is present. This non-specific response disappears on repeated application of the diluted blood, and in any case usually differs in form from that produced by solutions of ACh. In a few experiments (four out of fourteen), however, the first samples of fluid, collected at the beginning of the perfusion, showed an activity which could not be explained in this way, and which was doubtless due to the presence of ACh. The concentration of ACh. in such control samples, however, never exceeded 1×10^{-9}, or about 5 % of the concentration later found when the preganglionic nerve was stimulated. Later samples invariably failed to show even this slight activity. This small, temporary, and inconstant "spontaneous" output of ACh. is probably not due to injury discharge of impulses in the cut preganglionic trunk, since this was always divided about 2 hr. before. On the other hand, Lorente de Nó's suggestion, that this small initial output, when it occurs, is due to damage of the ganglion cells, has no real evidential basis. Brown & Feldberg's [1936*a*] evidence indicates that the ACh. extractable from the ganglion is located at the preganglionic nerve endings and dependent on their integrity; so that, if the ganglion were so injured in preparation as to liberate ACh., it would be natural to suppose that it would be liberated by the injury, as by nerve impulses, from the preganglionic nerve endings and not from the ganglion cells. On the other hand, after a dissection conducted so as to avoid directly touching the ganglion, and a long subsequent interval without eserine before the perfusion is begun, it seems little likely that even the traces of ACh. sometimes found in the early effluent could have been liberated by injury to the ganglion during dissection. While, however, the sympathetic ganglion itself is carefully avoided in dissection, a number of nerves and branches are necessarily cut in isolating the small mass of tissue, including both vagus and sympathetic ganglia, which is subsequently perfused. From the cut ends of these

nerves, retracted into the perfused tissues, it is likely that a small leakage of ACh. may continue for some time, and contaminate the early runnings of the eserinized perfusion fluid. In any case, this initial "spontaneous" output has, in my experiments, been so small and so evanescent as never to complicate the clear effect of preganglionic stimulation.

Release of acetylcholine by preganglionic stimulation

Stimulation of the preganglionic trunk never failed, in thirteen experiments, to cause the liberation of considerable quantities of ACh. (Fig. 1, Table I). The effect could be repeated, with short periods of stimulation, an indefinite number of times. Calculation of the quantity liberated per single maximal shock gave values lying remarkably close together. Thus in twenty-four out of twenty-six stimulations, the output per shock was between 0·000015 and 0·000035 μg., with a mean of 0·000024 μg.; in the other two cases the outputs per shock were 0·000008 and 0·000053 μg. respectively. These values are somewhat lower than those obtained by Feldberg & Vartiainen [1934], 0·000066 and 0·0001 μg., in two experiments made especially for the purpose of such estimates; I have also found values in the latter range, in experiments in which the perfusion fluid was eserinized Locke's solution rather than diluted blood. In any one of the experiments with diluted blood as perfusion fluid, the output per shock either remained nearly constant at all stages of the perfusion, or showed a slight falling off in the later stimulation periods. The variation, from one animal to another, of the quantity of ACh. liberated by each maximal stimulus, is scarcely greater, however, than the variation observed in the ACh. content of individual ganglia, and lends no support to the suggestion that the effectiveness of stimulation in liberating ACh. is proportional to the degree of injury suffered by the ganglion.

The transient relaxation of the contracted nictitating membrane during the initial period of a continuous, rapid, preganglionic stimulation, shown by Brown & Feldberg [1936c] to be due to the accumulation within the ganglion of an excess of free ACh., has been regularly observed in my experiments, when such a long-continued stimulation was applied.

Failure of vagus and postganglionic stimulation to liberate acetylcholine

Maximal stimulation of the vagus peripheral to the ganglion (in thirteen experiments), or of the postganglionic sympathetic nerves (in six experiments), failed without exception to cause the liberation of detectable quantities of ACh. (Fig. 1, Table I). Preganglionic stimulation

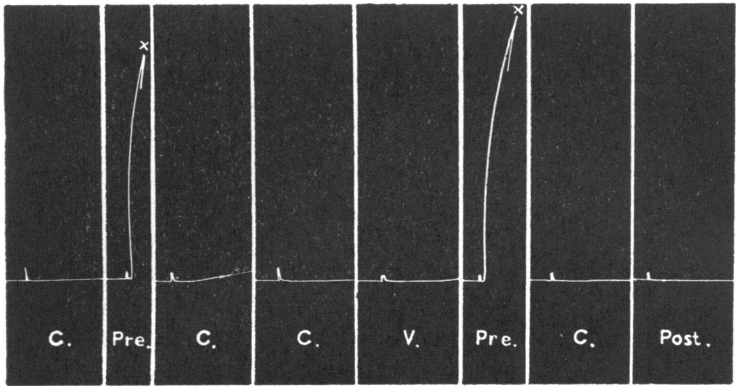

Fig. 1. Perfusion with diluted blood. Eserine 1 : 250,000. Perfusion rate 0·6 c.c. per min. Leech assay of successive 10 min. samples. *Pre.* = preganglionic stimulation, 9 min.; rest 1 min. *C* = control (no stimulation). *V* = vagus stimulation, 10 min. *Post.* = postganglionic stimulation, 10 min. Leech preparation sensitive to ACh. 5×10^{-10}. Change to clean Ringer solution at ×, with drum stopped.

TABLE I

Exp.	ACh. liberated (μg.) 1st control sample	Later control samples	Preganglionic stimulation	Postganglionic stimulation	Vagus stimulation
1	None	None	0·100 (8)	None (5)	None (8)
			0·083 (8)		None (8)
2	None	None	0·036 (8)	None (8)	None (8)
			0·078 (8)		
			0·084 (8)		
3	None	None	0·088 (8)	None (10)	None (10)
			0·092 (8)		
4	None	None	0·161 (8)	None (8)	None (8)
			0·115 (8)		None (8)
			0·110 (8)		
5	0·004	None	0·280 (8)	None (10)	None (8)
			0·170 (8)		None (10)
6	0·005	None	0·022 (1·5)	—	None (5)
			0·017 (1·5)		
7	None	None	0·027 (1·5)	—	None (5)
8	None	None	0·032 (3)	—	None (5)
9	0·003	None	0·085 (5)	—	None (5)
10	None	None	0·044 (4)	—	None (5)
11	None	None	0·040 (4)	—	None (5)
12	None	None	0·060 (4)	None (10)	None (8)
			0·051 (4)		None (10)
			0·096 (9)		
			0·100 (9)		
13	None	None	—	—	None (30)
14	0·002	None	0·050 (4)	—	—
			0·060 (4)		
			0·060 (4)		
			0·050 (4)		
			0·040 (4)		
			0·035 (4)		

The table summarizes the results of all the experiments in which the perfusion fluid was diluted blood. Figures in parentheses indicate the duration of stimulation in each case; the stimulation frequency was always 10 per sec. Values for ACh. outputs during control periods, where obtained, refer to 10 min. periods.

liberated ACh. in the usual amount in all these experiments, except No. 13, in which the cervical sympathetic nerve had been cut 3 days previously. In this case stimulation of this nerve caused no transmission of impulses through the sympathetic ganglion and no output of ACh. [see MacIntosh, 1938; Bacq & Coppée, 1938].

Absence of "delayed output" of acetylcholine

In most of the experiments the sample of perfusate corresponding to each stimulation period was collected during the progress of stimulation and a further 1 or 2 min. after its end. The succeeding sample was then completely inactive, or showed at the most a barely detectable trace of activity. In certain special experiments an attempt was made to determine more exactly how close a correspondence obtained between the

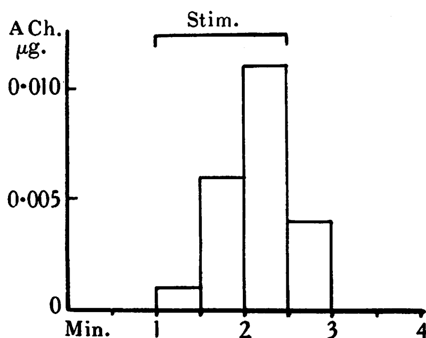

Fig. 2. Perfusion with diluted blood, eserine 1 : 250,000. Perfusion rate 1·2 c.c. per min. Rectangles indicate ACh. output in successive ½ min. periods. *Stim.* = preganglionic stimulation for 1½ min.

period of preganglionic stimulation and the period during which ACh. was present in the perfusate. For this purpose the venous effluent was collected in 30 sec. samples, which were separately assayed, the perfusion pressure being raised to 180 mm. Hg, in order to permit the collection of a sufficient volume of fluid in each period of this brief duration, and to reduce the effect of the dead space in the venous cannula, which had a volume of 0·08 c.c., and the collecting vein. The result of such an experiment is shown in Fig. 2. It will be seen that ACh. in detectable concentration is already present in the first half-minute sample collected during preganglionic stimulation; the output increases in succeeding periods, falls immediately in the 30 sec. following the end of stimulation, and in the next 30 sec. has returned to zero. With slower rates of perfusion,

traces of ACh. could occasionally be detected in samples collected during the second minute after the end of stimulation, but not in later samples.

The diffusion of liberated ACh. into the vessels of the ganglion, and its removal from these and from the outflow cannula, is thus complete within a period of from 30 sec. to 2 min., under different conditions. As Lorente de Nó has pointed out, it is obviously impossible to show, by such a method as this, whether or not there is *any* production of ACh. which outlasts the transmission of impulses through the ganglion. The present results, however, are certainly compatible with the opinion that there is not.

Absence of evidence of injury in the perfused ganglion

No sign of oedema could be detected by simple inspection, in any preparation perfused with dilute blood, even after perfusion had continued for several hours; in this respect there was a very marked contrast to preparations perfused with Locke's solution, in which the connective tissue surrounding the ganglion rapidly becomes distended with fluid. In three experiments, in each of which perfusion was continued for over 2 hr., the perfused ganglion and the control ganglion of the opposite side were weighed: the respective weights were 10·2 and 9·4, 11·9 and 12·3, and 12·1 and 11·3 mg. There was thus no significant increase in weight as a result of perfusion.

Through the kindness of Dr J. R. Perdrau, two ganglia, which had been perfused with diluted blood and shown to release ACh. as usual on preganglionic, but not on vagus stimulation, were examined histologically, and compared with control ganglia of the opposite side, both being excised after fixation with the minimum of dissection. Fixation was by Bodian's [1936] method, used by Lorente de Nó, which involves a short preliminary perfusion even of the control ganglion *in situ* with saline, and then of both with diluted alcohol. Sections were stained by Bodian's silver method, or with haemotoxylin-eosin, toluidine blue, or Weigart's fibrin stain. All sections showed considerable shrinkage of ganglion cells, to be attributed to the use of alcohol fixation; but in this and in all other respects there was no recognizable difference between the perfused and stimulated and the control ganglia. In particular, the perfused specimens showed little or no oedema of either the ganglion or its supporting tissue; mild leucocytic infiltration, involving the extracapsular tissue only, was observed in one of the perfused preparations, but also in one of the controls; blocked capillaries and fibrin were absent.

Perfusion with Locke's solution

In an attempt to find the reason for the discrepancy between the results of Lorente de Nó and those obtained in this laboratory, a number of perfusions were carried out with plain, eserinized Locke's solution. The effects of various perfusion pressures, ranging from 35 to 200 mm. Hg, and of pulsatile pressure (Dale-Schuster pump) as compared with static pressure (raised reservoir), were tested. These experiments, however, with one exception, gave results quite in accord with those obtained when the perfusion fluid was diluted blood. Preganglionic stimulation regularly caused the appearance of ACh. in the perfusate; vagus and postganglionic stimulation had no such effect. As might be expected, the removal of liberated ACh. by the perfusion fluid was somewhat slower at the lowest perfusion pressures: thus traces of activity, not exceeding 5 % of that present in "stimulation" samples, were under such conditions still detectable in samples of effluent collected in a period beginning 2 min. after the end of stimulation. A little ACh., not exceeding 2×10^{-9} in concentration, was sometimes present in the first control samples collected after the beginning of perfusion, but not in later samples prior to preganglionic stimulation.

In the one aberrant experiment the ganglion was perfused for 2 hr. with eserinized Locke's solution at the high, pulsatile pressure of about 200 mm. Hg. It became very oedematous, but still responded to preganglionic stimulation with a good output (0·00005 μg. per shock) of ACh. The pump was then disconnected, and perfusion continued from the reservoir at a static pressure of 40 mm. Hg, the perfusion rate falling to 15 % of its previous value. Preganglionic stimulation now failed to cause the appearance of any detectable ACh. in the perfusate, although there was a good response of the nictitating membrane; and further stimulation was equally ineffective in bringing out ACh., even after the original high, pulsatile pressure had been restored. This experiment is quoted, as being the single one in which, even under conditions made deliberately unfavourable, effective preganglionic stimulation was not accompanied by demonstrable release of ACh. from the ganglion. It is to be noted that, after such deliberate distension of the tissues by high pressure, the departure from the normal result, with subsequent slow perfusion, was not the appearance of ACh. without stimulation, but its failure to reach the effluent when the preganglionic nerve was stimulated. In another experiment, in which exactly the same sequence was applied, preganglionic stimulation caused the normal appearance of ACh. in the

effluent at all stages of the perfusion. The most probable explanation of this one case, in which no discharge of ACh. could be detected, is the occurrence, with the change from high pulsatile to low static pressure, of a localized obstruction, causing the perfusion thereafter to pass chiefly through the tissues outside the sympathetic ganglion. Earlier experience in this laboratory had shown the necessity of great care with regard to filtration of the perfusion fluid, particles of microscopic size being liable, by blocking small arteries, to produce retardation and irregular distribution of the flow through the perfused tissue. It is not difficult to imagine that an abrupt and wide variation of the perfusion pressure might cause the flow thus to be largely diverted from the ganglion itself.

Acetylcholine in the venous blood of a stimulated ganglion

Feldberg & Vartiainen [1934] observed that preganglionic stimulation in the eserinized cat may lead to the appearance of ACh. in the venous blood coming from the ganglion. I have made a few similar experiments, but in this case have obtained varying results. The ganglion was isolated as for perfusion, but retained its natural blood supply from the common carotid; atropine and massive doses of eserine (2–6 mg. per kg.) were given intravenously, and the animal's blood was made incoagulable with adequate doses of heparin. The blood flow through the preparation was always extremely slow (1 c.c. in 12–40 min.), and samples usually showed slight activity in the absence of stimulation. In three out of five experiments, preganglionic stimulation failed to increase the ACh. concentration of the venous blood, although it could be shown that the blood contained sufficient eserine to preserve ACh. In the other two experiments, preganglionic stimulation regularly produced a clear-cut augmentation of the ACh. output; in one of these the vagus was stimulated, without producing any effect. In the experiments yielding positive results, the output of ACh. per shock lay between 0·000002 and 0·00001 μg., being thus well below that found in the perfusion experiments. Feldberg & Vartiainen only record one experiment made with natural circulation, and in this the concentration of ACh. in the venous blood from the ganglion rose during preganglionic stimulation to 1 in $2{\cdot}5 \times 10^7$, which was within the limits of concentration obtained, during similar stimulation, in a perfusion effluent. Since, however, blood flows from the venous cannula, with natural circulation, very much more slowly than the fluid with artificial perfusion, they clearly obtained a correspondingly less rapid output of ACh. Substantially, therefore, my positive results confirm theirs; but my general experience shows, in addition, that the

appearance of ACh. in the venous outflow from the ganglion, as a result of preganglionic impulses, is not always to be detected when the ganglion retains its natural circulation.

Discussion

The result of my experiments is to confirm those of Feldberg & Gaddum, and particularly those of Feldberg & Vartiainen. In contrast to the experience recorded by Lorente de Nó, I have not observed any continued or significant output of ACh. from the perfused ganglion without stimulation; I have never failed to observe its prompt appearance in response to, and in satisfactory coincidence with, periods of stimulation of the preganglionic nerve; and I have never detected any output during stimulation of either the vagus trunk or the sympathetic post-ganglionic branches, or in the periods succeeding such stimulation. In general, in my experiments with artificial perfusion of the ganglion, ACh. has regularly appeared in the venous effluent when the preganglionic nerve was stimulated, and only then. It failed so to appear only in one case, in which special measures had been taken to disturb the regular course of the perfusion. When the ganglion and the surrounding tissues have become oedematous, local variations in the resistance to perfusion might change the distribution of the flow, so that at one time the surrounding tissues, and at another those of the ganglion itself, might make the predominant contribution to the venous outflow; and one might expect such a changing incidence of the main perfusion stream to be more prominent with a slow perfusion at low pressure. It seemed possible that irregularities of this kind might be concerned in the capricious results recorded by Lorente de Nó, especially as he appears to have perfused under lower pressures than have generally been used by me, and by my predecessors in this line of investigation. My own attempts to produce such irregular conditions, however, have not on the whole been successful. In the one case mentioned, the output of ACh. failed when the pressure and perfusion rate were suddenly reduced, but in other cases I still observed a close correspondence between its appearance and the periods of preganglionic stimulation, even when pressures lower than those recorded by Lorente de Nó were employed. My difficulty, indeed, has been, not to obtain such coincidence, but to find conditions leading to a delayed or irregular output of ACh., or to its appearance with stimulation applied to nerves other than the preganglionic, as described by Lorente de Nó.

If Lorente de Nó's attribution of the release of ACh. to injury of the ganglion were correct, it would be expected that perfusion with diluted blood instead of simple Locke's solution, and the consequent avoidance of any significant oedema, would be unfavourable to its regular appearance. My use of this method shows, on the contrary, that these conditions produce a remarkable uniformity, in different experiments and at different stages in the same experiment, of the amounts of ACh. liberated by the same number of preganglionic volleys. A ganglion so perfused shows, further, no significant difference from the corresponding normal ganglion, when examined histologically at the conclusion of an experiment. I did not, indeed, employ the same elaborate precautions as Lorente de Nó to avoid slight cooling or drying of the ganglion during the preliminary dissection. On the other hand, I regularly completed the dissection in 30 or 40 min., with care to avoid direct manipulation of the ganglion, in comparison with the 4–5 hr. required by Lorente de Nó for dissection under the microscope, in those experiments in which he attributed the smallness of the release of ACh. to the preservation of practically normal conditions in the ganglion.

My results thus give no support to the suggestion that the release of ACh. from the perfused ganglion is due merely to injury, apart from the arrival of preganglionic impulses at the synapses. On the other hand, they do not justify the assumption that the ACh. liberated at the synapses can escape, under the protection of eserine, into the blood circulating through the vessels of a ganglion which is otherwise perfectly normal. When the ganglion was allowed to retain its natural circulation, I detected the appearance of ACh. in the venous blood in response to preganglionic stimulation in only two out of five experiments, and then in relatively small amounts. One experiment of this kind was recorded by Feldberg & Vartiainen [1934] as evidence that the liberation could occur with natural blood supply, but a series would have been needed to show that it does so regularly; and, in fact, Dr G. L. Brown informs me that in subsequent experiments of this kind, which he made with Dr Feldberg in another connexion, positive results were not obtained in all cases, in conformity with my own findings. It is perhaps significant also that, in the largely analogous case of the voluntary muscle responding to motor nerve impulses, Dale *et al.* [1936] failed to detect any ACh. in the venous blood when the muscle retained its natural circulation under eserine, though it always appeared in the venous effluent with saline perfusion. It may, therefore, be regarded as not improbable, that some degree of departure from perfectly normal conditions may be required to

enable ACh., liberated at the synapses in the ganglion, to escape into the circulation so as to be detected in the fluid leaving the vein. It may be that eserine, in doses tolerable by the whole animal, fails completely to inactivate the cholinesterase concentrated at the normal synapses [cf. v. Brücke, 1937]. On the other hand, as Feldberg & Vartiainen [1934] point out in discussing the action of eserine, the function of the cholinesterase in the ganglion may be to prevent the diffusion of excess of ACh. away from the point of its liberation at the synapse, rather than to remove it, by destruction at the site of its release, during the refractory period of the ganglion cell. There may be some other mechanism for such removal, effective under completely normal conditions. Normally most of the liberated ACh. may be immediately refixed in the inactive complex from which an impulse has released it; and the recent description by Mann *et al.* [1938] of the aerobic synthesis of an "acetylcholine-precursor" by brain tissue, suggests a method by which this may occur. Some degree of failure of normal oxygenation, or some increase of the permeability of the tissue above the normal, insufficient in either case to interfere with the transmission of excitation at the synapse, may, then, be necessary to enable the liberated ACh. to appear in the venous effluent in a quantity sufficient for detection and measurement. The question at issue, however, is not whether such slight abnormality is necessary to enable the liberation of ACh. to be detected, but whether, as Lorente de Nó appears to suggest, it is in itself the sufficient cause of such liberation. In my experiments it has not been so. The slight abnormality due to perfusion, if it exists, is one which can be regularly reproduced; it does not by itself lead to the appearance of ACh. in the venous fluid in significant amounts; it simply creates the conditions under which ACh. always appears in the effluent when preganglionic impulses reach the synapses, but never in their absence.

Lorente de Nó's experiments were made to control certain of those made by Feldberg & Gaddum [1934] and Feldberg & Vartiainen [1934], and his discussion deals essentially with the differences between his results and theirs, and his consequent doubt of the significance of their findings for the theory of transmission of excitation by ACh. at ganglionic synapses. The case for that theory cannot, however, be fairly assessed without consideration of additional points of evidence, of which Lorente de Nó's argument takes no account, and with which, indeed, it is at some points inconsistent. Brown & Feldberg [1936*a*] found that degeneration of the preganglionic fibres causes the disappearance of nearly all the ACh. which the normal ganglion yields to extraction, and that, after

such degeneration, the perfused ganglion does not, like the normal ganglion, liberate ACh. in response to K ions, though these still effectively stimulate the ganglion cells. More recently [MacIntosh, 1938] I have shown that this disappearance of ACh. takes place in the 3 days following preganglionic section, in close accordance with the failure of transmission of excitation through the ganglion, at a time when, as Bacq & Coppée [1938] showed in parallel experiments, conduction in the preganglionic fibres is still maintained. At a time, therefore, when both the preganglionic fibres and the ganglion cells appear to be functionally intact, acetylcholine has almost disappeared from the ganglion and transmission across the synapses no longer occurs. Brown & Feldberg [1936*c*] further found that a normal ganglion will discharge up to five times its initial store of ACh. during continuous stimulation by preganglionic volleys for some hours, and still retain its original store undiminished. The selective action of certain poisons such as curare [Brown & Feldberg, 1936*b*] in rendering the ganglion cells completely insensitive to ACh. and to preganglionic impulses, while leaving them normally responsive to stimulation by K ions, must further be taken into account. It would be difficult to bring any of this evidence into intelligible relationship with Lorente de Nó's suggestion, that the ACh. liberated from a perfused ganglion is a product of the metabolism of, or of injury to, the ganglion cells, having no connexion with the transmission of excitation at the synapses.

SUMMARY

1. A method is described for the perfusion of the superior cervical ganglion with diluted blood.

2. Ganglia so perfused remain in excellent physiological condition and present no evidence of damage on histological examination.

3. Such ganglia do not liberate ACh. spontaneously, or on stimulation of the vagus or of the postganglionic sympathetic fibres: but ACh. is regularly liberated from them on stimulation of the preganglionic nerve. The liberation of ACh. begins with such stimulation, and ceases promptly after it.

4. The original findings of Feldberg & Gaddum and of Feldberg & Vartiainen are thus confirmed, and the significance in relation to them, of other evidence for the chemical transmission of excitation in the ganglion, is discussed.

I am deeply grateful to Sir Henry Dale for his stimulating interest in this work. My thanks are also due to Dr G. L. Brown for much valuable advice.

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