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ACETYLCHOLINE RELEASE IN THE CAT'S SUPERIOR CERVICAL GANGLION

By W. L. M. PERRY

From the National Institute for Medical Research, Mill, Hill, London, N.W. 7

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The release of acetylcholine at sympathetic ganglionic synapses during preganglionic stimulation was first demonstrated in 1934 by Feldberg & Gaddum on the perfused superior cervical ganglion of the cat. Since then a number of workers, notably Feldberg & Vartiainen (1934), Brown & Feldberg (1936), and MacIntosh (1938), have confirmed and extended the observations. None of these workers, however, made a detailed quantitative study of the amounts of acetylcholine released under different experimental conditions; they confined their observations mainly to demonstration of the fact that, in the cat's eserinized perfused superior cervical ganglion, the output of acetylcholine during preganglionic stimulation began relatively high and fell in an approximately exponential fashion finally to reach a fairly steady level.

Furthermore, since all these experiments were carried out in the presence of eserine, it was always tacitly assumed first, that the output of acetylcholine would be the same whether the released acetylcholine was destroyed or not, and secondly, that eserine itself had no effect on the output of acetylcholine.

In the present experiments the output of acetylcholine, during long periods of preganglionic stimulation at different frequencies, has been studied quantitatively and an attempt has been made to interpret the results on the assumption that the rate of synthesis of acetylcholine is constant, and that the amount of acetylcholine liberated by a single preganglionic volley is a constant fraction of the stock of acetylcholine available for release at the time.

In addition, an attempt has been made to find out if eserine influences the release of acetylcholine during prolonged preganglionic stimulation. This problem has been tackled in two ways. In the first place the effect of eserine on the recovery of the acetylcholine released during a period of rest after prolonged stimulation has been studied; in the second place a comparison has been made during preganglionic stimulation between the output of acetyl-

choline in the presence of eserine and the output of choline in the absence of eserine. It has been shown in these experiments that eserine appears to exert a considerable influence on the release of acetylcholine.

METHODS

Cats were anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (80 mg/kg). The superior cervical ganglion was prepared for perfusion by the method described by Kibjakow (1933) with the modifications suggested by Feldberg & Gaddum (1934). In addition, the perfusion fluid, instead of being warmed in an electrically heated cannula, was warmed by passing the inflow tube through the cat's oesophagus from below upwards, so that its tip emerged at the point where the common carotid artery was cannulated; and the perfusion fluid consisted of Locke's solution containing twice the usual amount of glucose (making a final concentration of 2 g/l. of glucose). These modifications were suggested to me by Dr F. C. MacIntosh, who had used them in previous experiments. When desired, eserine was added to the perfusion fluid to make a final concentration of 1 in 100,000. This concentration has been used previously in similar experiments, although a concentration of 1 in 200,000 has, perhaps, been more usual.

The preganglionic cervical sympathetic trunk was stimulated with square waves of 0·5 msec duration, the stimulus strength being supramaximal for the contraction of the nictitating membrane. Except where specifically stated otherwise, the frequency of stimulation used was 10 shocks per sec.

In some experiments the choline output of the uneserinized perfused ganglion was measured. For this purpose the choline in the perfusate was acetylated, as first described by Guggenheim & Loeffler (1916), and was then assayed as acetylcholine. For the details of the method of acetylation the description of Fletcher, Best & Solandt (1935) was followed with the minor change that, as clear perfusates instead of tissue extracts were being acetylated, it was possible to reduce the time necessary for full acetylation from 2 hr to 5 min. This was checked in a control experiment, the results of which are shown in Table 1.

TABLE 1. Recovery of choline in acetylation process

Sample no.	Weight of choline chloride (mg)	Time of acetylation (min)	Weight of acetylcholine recovered, assayed on frog rectus (mg)
1	19·6	180	20
2	17·0	20	16·6
3	19·8	5	20

Acetylcholine was assayed on the blood pressure of the eviscerated chloralosed cat as described in detail by MacIntosh & Perry (1950).

RESULTS

Output of acetylcholine from the eserinized ganglion

The output of acetylcholine during stimulation of the eserinized perfused ganglion has been shown to fall approximately exponentially (Feldberg & Vartiainen, 1934; Brown & Feldberg, 1936; MacIntosh, 1938). At the start of stimulation, when the output is falling rapidly, the shorter the period of collection, the higher are the initial concentrations of acetylcholine, and this is true until the period of collection is so short that the acetylcholine liberated is not carried out into the collecting vessel within the period, but owing to the

limited rate of flow, is carried over into the following sample. When the initial collection periods were reduced to 30 sec (Fig. 1*a*) the output rose in the second period before starting to fall exponentially; this result is similar to one reported by MacIntosh (1938). In Fig. 1*b* the output of acetylcholine per 2 min sample in one experiment is shown (solid lines). If, owing to a slow flow of the perfusion fluid, one third of the amount of acetylcholine liberated

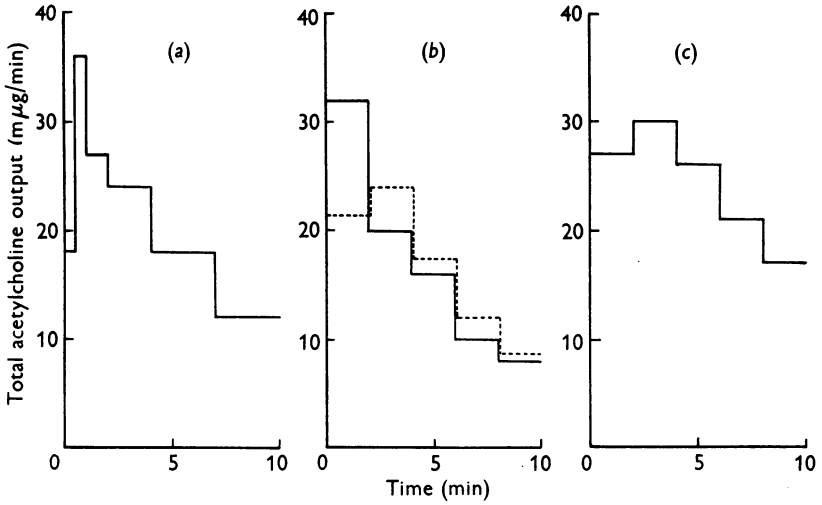

Fig. 1. Histograms of output of acetylcholine from perfused eserinized superior cervical ganglion. (*a*) Effect of reducing periods of collection of output to 30 sec (flow rate 0·8 ml. per min). (*b*) Effect of slowing rate of flow; continuous lines: outputs per 2 min period; dotted lines: theoretical outputs per 2 min period if one-third of total output from ganglion in each period is carried into next sample owing to slow flow. (*c*) Outputs per 2 min period with flow of 0·3 ml. per min. (For details see text.)

in each period had been carried over into the following period, the result would be the outputs of acetylcholine illustrated graphically (dotted lines). Thus in this case also—i.e. where the flow is very slow—the result is a rise in output in the second, and occasionally even in the third period; an experiment in which this actually was observed in 2 min periods of collection is shown in Fig. 1*c*.

In order to avoid these initial rises in output, collection periods of 2 min duration were usually used, and this was found to be successful in its object, provided that the rate of flow was of the order of 0·5 ml. per min at least.

Frequency of stimulation. The output of acetylcholine was followed during 40 min stimulation at frequencies of 5, 10, 20, 31 and 100 per sec, samples being collected over 2 min periods. Fig. 2 shows the exponential fall in output observed in one experiment at a frequency of 10 per sec, and a similar experiment by Brown & Feldberg (1936) at a frequency of 15 per sec and collection for periods of 5 min.

The outputs of acetylcholine for different frequencies of stimulation, obtained in different experiments, are shown in Fig. 3. The curves obtained are substantially the same in each case; the output of acetylcholine per min falls at about the same rate and to about the same final steady level of 4 mμg per min whatever the frequency of stimulation. There is thus no evidence of any greater reduction in the acetylcholine output at the higher frequencies,

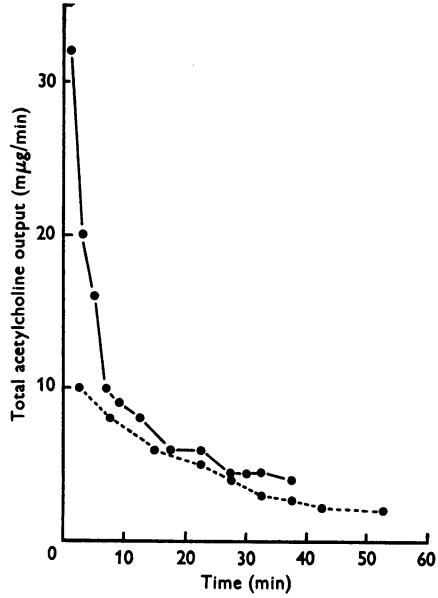

Fig. 2. Graph of output of acetylcholine from perfused eserinized superior cervical ganglion. Continuous line: experiment with periods of collection of 2 min. Dotted line: experiment from Brown & Feldberg (1936) with periods of collection of 5 min.

in spite of the fact that, when the frequency is more than 30 per sec the preganglionic terminals may well fail to respond adequately. Therefore the amount of acetylcholine liberated per volley must be considerably reduced at higher frequencies of stimulation; and this reduction must occur from the same initial output level for the first volley, since the ganglion cannot anticipate the frequency of stimulation from the initial shock. Thus the rate of deterioration in output per volley must be much more rapid for the higher frequencies of stimulation. The amounts of acetylcholine liberated per volley for different stimulus frequencies are plotted on a logarithmic scale against time in Fig. 4.

The fact that the final steady level of output of acetylcholine of about 4 mμg per min is constant for all frequencies of stimulation could be taken to imply that, under the conditions of eserinized perfusion, this is the rate at which the ganglion synthesizes acetylcholine; while the initial high rates of

output could be held to imply the release of some available stock of acetylcholine already held in the ganglion. If these premises were correct, the release of acetylcholine might take place according to some definite pattern.

For instance, if we assume that any one preganglionic volley will liberate a constant fraction (α) of the total acetylcholine available in the ganglion at

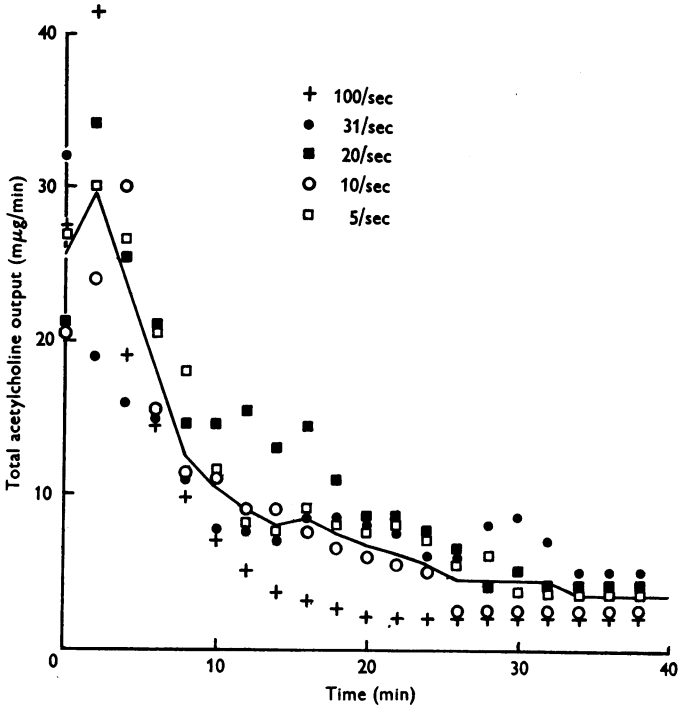

Fig. 3. Graph of output of acetylcholine from perfused eserinized superior cervical ganglion at various rates of stimulation. Continuous line: mean of all experiments.

the moment, and that the rate of synthesis by the ganglion (K) is a constant independent of the rate of stimulation, then the stock of acetylcholine available for release after a definite time (t) may be written as

$$y_t = y_0 \, e^{-\alpha t} + \frac{K}{\alpha}(1 - e^{-\alpha t}),$$

where y_0 is the initial stock of acetylcholine in the ganglion and available for release. In Fig. 5 the results of one experiment, at a stimulus frequency of 10 per sec, are shown to fit this hypothesis without serious discrepancy. The equation of the fitted curve is, of course,

$$\alpha y_t = K(1 - e^{-\alpha t}) \quad + \quad \alpha y_0 \, e^{-\alpha t},$$

since αy is the amount liberated per volley. Fitted values for α and K in this case of

$$\alpha = 0{\cdot}0005,$$

$$K = 2{\cdot}0 \text{ m}\mu\text{g per min},$$

were used: a χ^2 test between the observed and fitted values of αy_t gives $\chi^2 = 4{\cdot}40$ on 9 degrees of freedom, so that the fit is satisfactory at the $P = 0{\cdot}05$ level of probability.

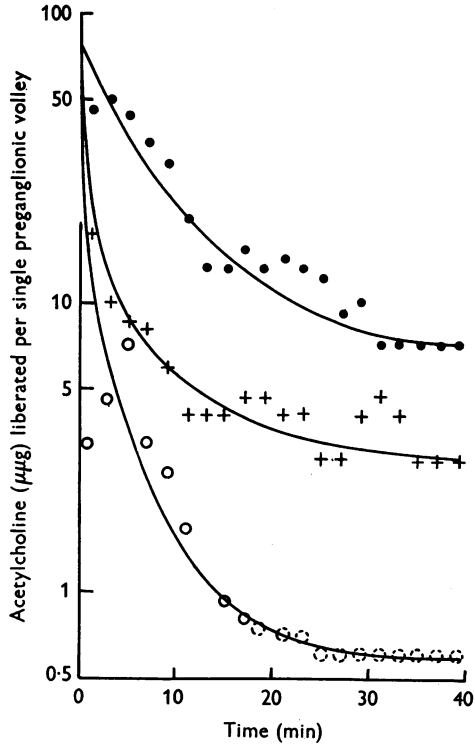

Fig. 4. Graph of output of acetylcholine from perfused eserinized superior cervical ganglion at stimulation frequencies of (●—●), 10 per sec; (+—+), 31 per sec; and (○—○), 100 per sec. The dotted circles represent values estimated in an assay where the total amount of acetylcholine was so small that the error of the estimate was very large.

The values of y_0, i.e. the original stock of acetylcholine available in the ganglion estimated by this method, fall between 200 and 250 mμg, so that with $\alpha = 0{\cdot}0005$ the initial output per volley falls in the range 100–125 $\mu\mu$g. This figure is independent of the rate of stimulation, and is in good agreement with the figures given by Feldberg & Vartiainen (1934), who stimulated at a rate of approximately 2 per sec for very short periods and obtained outputs of 66 and 100 $\mu\mu$g per volley in two experiments.

If this theoretical explanation of the output per volley has any truth, we would not expect that the outputs per min at different rates of stimulation would follow exactly the same pattern: but they would fall into patterns so similar that the differences between them would not be much greater than those observed, which are well within the experimental errors of the method.

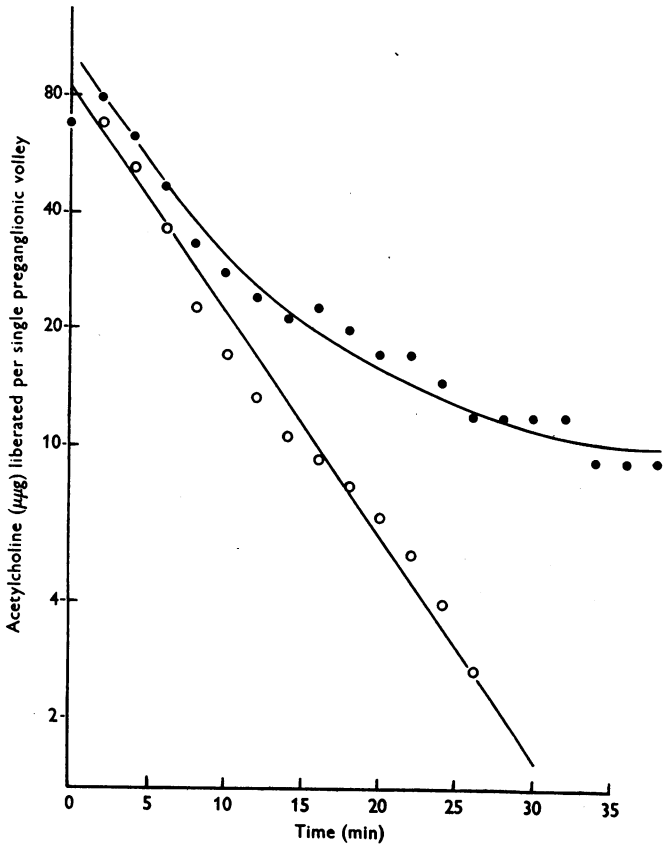

Fig. 5. Graph of output of acetylcholine from perfused eserinized superior cervical ganglion stimulated at 10 per sec. (●—●), observed output of acetylcholine; (○—○), output of acetylcholine calculated on the basis that no synthesis occurs.

Recovery of acetylcholine output during rest periods. It was found that a period of rest of 15–20 min between two periods of prolonged stimulation did not restore the rate of output of acetylcholine in the second period to its initial level in the first period. The results of five such experiments are illustrated in Table 2. In each experiment a 40 min period of stimulation was followed by a rest period of varying duration, and stimulation was then restarted at the same frequency. The acetylcholine output was determined on

2 min samples both before and after the rest period. The maximal outputs of acetylcholine per min during the first and the second period of stimulation are given in columns 3 and 7. Usually the maximal rate occurred in the first sample, except when the rate of flow was very slow. In column 10 the duration in minutes of the period of rest between the two periods of stimulation is given. It will be seen that, even after 35 min rest, the maximum output had risen to

TABLE 2. Effect of rest periods on output of acetylcholine

	Observed output of acetylcholine in mμg			Calculated acetylcholine in mμg		Observed maximum output of acetylcholine after rest period (mμg/min)	Time in min of rest period: Calculated as: Necessary to replace stock	Necessary to give observed max. output/min after rest	Given in each expt.
Expt.	Total in 40 min	Maximum/min	Final/min	Synthesized in 40 min	Lost from initial stock				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	437	32	4	160	277	6	69	12	5
2	406	41	3	120	286	9	25	20	25
3	408	32	4	160	248	—	62	—	—
4	613	34	6	240	373	24	62	47	35
5	351	30	3	120	231	6	77	16	20

only 24 mμg per min, although the maximal output in the first period of stimulation was 34 mμg per min, i.e. to 70 % of the original value. To some extent this difference may be attributable to a general deterioration in the condition of the preparation, since after 2 hr perfusion with Locke's solution, the ganglion has usually become oedematous.

Neglecting this factor, however, and knowing the amount of acetylcholine released during the first 40 min period of stimulation, we can calculate the amount of acetylcholine produced during this period by the assumed constant rate of synthesis; namely by multiplying the final steady level of output of acetylcholine per min reached during the first stimulation period by 40. The final steady rate of output per min is shown in column 4, and the total synthesis in the first 40 min period of stimulation in column 5. By subtracting this value from the total output of acetylcholine during the first stimulation period (column 2), we get an estimate (column 6) of the loss of acetylcholine from the stock originally present in the ganglion. From the five experiments the mean value for this loss is 283 mμg, and it may well be significant that this figure closely approximates to the total amount of extractable acetylcholine in the ganglion, which, according to Brown & Feldberg (1936), averages 250 mμg. Their statement that this figure was exceeded by the total amount of acetylcholine liberated during the period of exponential fall is also compatible with the present finding.

Further, if we assume that the constant rate of synthesis which accompanies

stimulation is maintained but not increased during the rest period, we can calculate the total synthesis that should have occurred during rest; and we can, by interpolation on a graph such as that shown in Fig. 5, arrive at an estimate of the time of rest which would theoretically be necessary to restore the output to the level observed at the start of the second period of stimulation. For instance, the figure 12, given for the first experiment in column 9 of Table 2, was obtained in the following way. The initial stock of acetylcholine available for liberation was 277 mμg, and 65 mμg were liberated and 8 mμg synthesized in the first 2 min; so that after 2 min stimulation 220 mμg were available for release. In the second 2 min period X mμg were found to be released and therefore, had X mμg been released at the start of the second stimulation period, the stock of acetylcholine at that time would, by inference, be 220 mμg. Thus, at a rate of synthesis of 4 mμg per min, it would take 220/4 min, i.e. 56 min rest to attain the observed maximum output of X mμg per 2 min. By a continuation of this process, the time necessary can be calculated for any value of the maximum output in the second period of stimulation. The times calculated in this way for each experiment (column 9) and the periods of rest actually given (column 10) agree fairly well.

There are several reservations to be made before drawing any conclusion from these findings. First, the experimental error in determining the rate of synthesis is large, since the assay of the very small amounts of acetylcholine in the later samples of a perfusion is difficult and has wide limits of error. In the second place, the times calculated as necessary to replace the whole of the lost stock of acetylcholine in all five experiments are of the order of 1–1½ hr, as seen in column 8. However, Brown & Feldberg (1936), on removing the ganglion shortly after prolonged stimulation, found no apparent reduction of the extractable acetylcholine, and MacIntosh (1938) was able to demonstrate a loss of up to 50 %, only by taking extreme measures to prevent synthesis occurring during the very short time between the end of stimulation and the extraction of the ganglion.

Effect of stimulation in the absence of eserine on subsequent stimulation in the presence of eserine

It has been shown that, when eserine is present throughout, the acetylcholine output in a second period of stimulation after a period of rest of 10 min starts at a greatly reduced level. This 'fatigue' of the ganglion was not found to occur when the first period of stimulation took place in the absence of eserine. If the initial stimulation was carried out with the normal blood supply to the ganglion left intact, or during perfusion with non-eserinized Locke's solution, the acetylcholine output of the second period of stimulation during perfusion with eserinized Locke's solution showed no such reduction. In all these experiments the rate of stimulation was 10 per sec, the length of

the first stimulation period was 40 min, and of the period of rest was 10 min. A typical experiment is illustrated in Fig. 6. Expt. 1(*a*) and (*b*) shows the outputs of acetylcholine during the first and second period of stimulation respectively, when the ganglion was perfused throughout with eserinized Locke's solution. Expt. 2(*b*) shows the output of acetylcholine in the second period of stimulation, when the ganglion was perfused with eserinized Locke's solution. In Expt. 2, however, the first stimulation period was carried out in the absence of eserine, and thus no figures are available for the acetylcholine

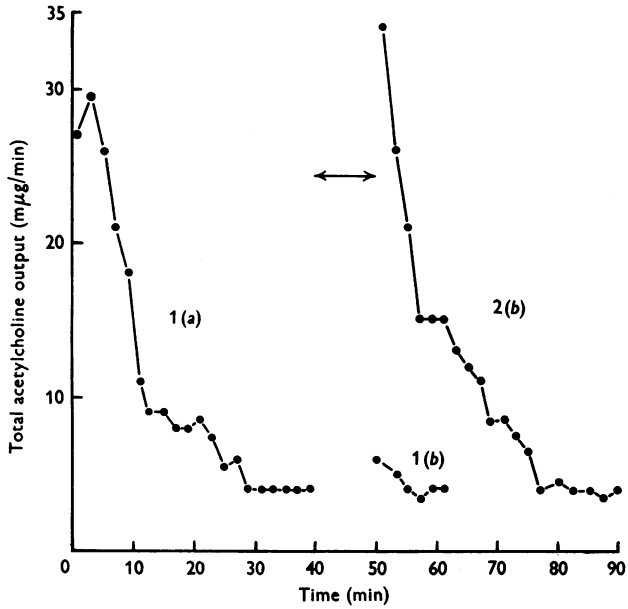

Fig. 6. The effect of eserine on recovery of superior cervical ganglion from stimulation at 10 per sec. 1 (*a*) and (*b*) eserinized throughout. 2 (*b*) eserinized during 10 min rest after 40 min stimulation of uneserinized perfused ganglion. Rest period: 10 min.

output at this stage. Expt. 2(*a*) was an initial stimulation period performed during perfusion without eserine, and during the period of rest the perfusion fluid was changed to eserinized Locke's solution. In another experiment an initial stimulation was performed with the blood supply to the ganglion still intact, cannulation being carried out during the 10 min rest. The outputs in the second period of stimulation were very similar to those of Expt. 2(*b*).

In order to discover whether eserine alone, in the absence of stimulation, would cause a reduction in the output of acetylcholine once stimulation was started, the ganglion was perfused with eserine for 40 min before stimulating; no reduction in the output was detectable under these conditions. Moreover,

the time between starting the perfusion and starting the stimulation was varied from experiment to experiment and no correlation between this delay and the output of acetylcholine was demonstrable.

Output of choline from the unstimulated perfused ganglion

When determining the output of choline by acetylating the perfusate, the figures obtained naturally include any acetylcholine present. But, since no acetylcholine is released from the unstimulated ganglion, the values can be taken as reliable figures for choline output.

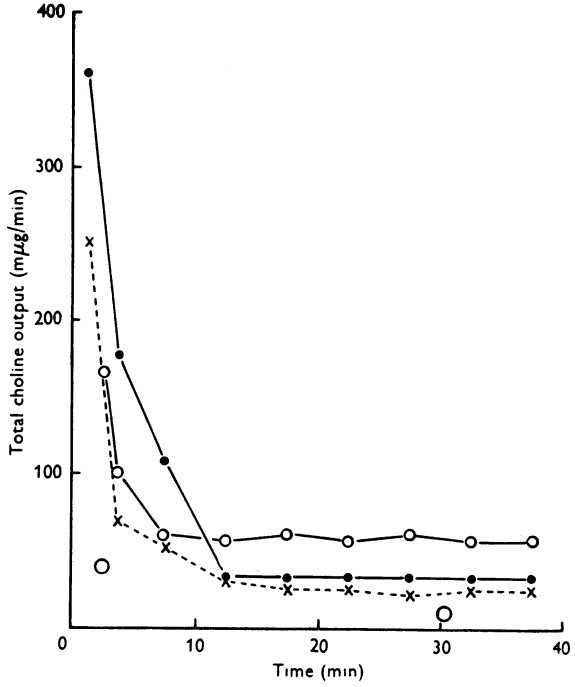

Fig. 7. Graph of output of choline from the unstimulated perfused superior cervical ganglion. Three experiments.

The observation of MacIntosh (personal communication) that perfusate obtained from a perfused, unstimulated ganglion contains considerable quantities of choline was confirmed. At the start of perfusion the choline output was high and varied from 100 to 400 mμg of choline per min (Fig. 7). This big variation is attributable to the time taken between the beginning of the perfusion and the collection of the first sample of effluent; if there is any delay in cannulation of the vein, the initial large concentrations of choline pass back into the animal's venous blood.

In all experiments the output of choline fell sharply and became steady within 15–30 min, at a level varying from 20 to 60 mμg per min in different experiments, but in any one experiment being quite well defined within narrow limits. The choline in the perfusate may be derived not only from the sympathetic ganglion but also from the stump of the vagus nerve and the nodose ganglion, and parts of the glossopharyngeal and accessory nerves which are included in the perfused tissue. It seems probable that the initial high choline levels are due to a washing out of choline from these structures. The fact that the output of choline becomes steady is more difficult to explain, but may be due to continuous metabolic changes in the perfused tissues; in any case it enables measurements to be made of the effect of stimulation on the choline output.

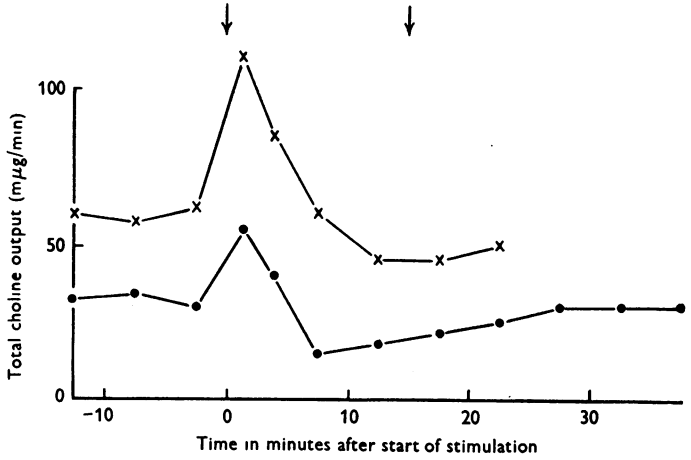

Fig. 8. Graph of output of choline from perfused superior cervical ganglion stimulated at 10 per sec between signals. Two experiments.

Output of choline from the stimulated perfused ganglion

In order to measure directly the output of acetylcholine in the absence of eserine, it is necessary to assume that the extra choline liberated by stimulation in the absence of eserine derives solely from the destroyed acetylcholine. Stimulation invariably increases the output of choline by about 20–50 mμg per min (Fig. 8). This corresponds to the amount of acetylcholine found during the initial stimulation of an eserinized preparation. MacIntosh (personal communication) was also able to obtain a release of choline of this order of magnitude during stimulation. The increased output of choline, however, is maintained for a few minutes only, and after about 5 min the choline output falls below the original resting level, even if stimulation is maintained. The fall below the resting level was slight, but was regularly obtained. At the end of a stimulation period the output may rise again to attain its original resting level.

DISCUSSION

The results obtained confirm the findings of Brown & Feldberg (1936) that the output of acetylcholine from the stimulated, eserinized, perfused ganglion falls approximately exponentially. The course of this fall has been traced in rather more detail, since the periods of collection of samples were reduced from 5 or 10 to 2 min. This has enabled the early part of the time-course of the output to be followed sufficiently closely to estimate the initial level of output at the first preganglionic volley. It was found that the first volley would be expected to liberate 100–125 $\mu\mu$g of acetylcholine, and this figure is in good agreement with that suggested by Feldberg & Vartiainen (1934) on different grounds.

The hypothesis has been advanced that each preganglionic volley liberates a constant fraction of the available stock of acetylcholine, and that there is a fixed rate of synthesis of acetylcholine which is constant whether or not the ganglion is stimulated preganglionically and whatever the frequency of stimulation may be. Although at frequencies of 30 and 100 per sec the preganglionic terminals may fail to respond adequately all the results obtained at a variety of frequencies of stimulation fit this hypothesis. Moreover, the recovery in output during periods of rest following prolonged stimulation was found to occur at a rate which also fits in with the assumed constant rate of synthesis—e.g. after 40 min stimulation, full recovery in output to the original level did not occur even when the rest period given was 35 min.

But there is one fact that is not in agreement with the hypothesis in its present form; both Brown & Feldberg (1936) and MacIntosh (1938) found that the replacement of any loss which might have occurred during prolonged stimulation in the extractable acetylcholine of the ganglion was very rapid indeed. In fact it required considerable care in extracting the ganglion as quickly as possible after stimulation in order to observe any loss at all. This finding implies a very much more rapid rate of synthesis, at least during periods of rest, than the present hypothesis indicates, and the discrepancy cannot easily be explained. The possibility exists that the synthesis of acetylcholine occurring during rest at a very rapid rate does not provide 'available' acetylcholine in the sense that it cannot be at once released by further stimulation.

The failure of the ganglion to return to its original level of output of acetylcholine, even after long periods of rest, might be attributable to a general deterioration in the preparation over such a long period of perfusion, or to a non-specific effect of eserine (e.g. Holton & Perry, 1951). But eserine alone does not produce such an effect. Perfusion with eserine in the absence of stimulation does not reduce the amount of acetylcholine liberated by stimulation. It is the two factors eserine and stimulation, together, and not either

alone, which causes the reduction in output. Dr W. Feldberg has suggested that in the presence of eserine, the released acetylcholine persists near the nerve terminals for a longer period and that the acetylcholine itself might block subsequent impulses in the nerve terminals. It proved impossible, however, to demonstrate any reduction in the amount of acetylcholine liberated by stimulation from a ganglion which had been previously perfused with Locke's solution containing eserine 10^{-5} and acetylcholine 10^{-6} for 40 min in the absence of stimulation. It therefore seems unlikely that this explanation is the correct one.

The experiments on the choline output of the ganglion are of theoretical interest. If the amount of choline released had remained high throughout the period of stimulation, it would have been reasonable to infer that this choline represented the destroyed acetylcholine released by stimulation. Therefore, in the absence of eserine, the release of acetylcholine during stimulation would presumably also have remained high, and the fact that it fell off in an exponential fashion in the presence of eserine would suggest that eserine exerted an inhibitory effect on the synthesis of acetylcholine.

If, on the other hand, stimulation had not increased the choline output at all, then the fact that in the presence of eserine increased amounts of acetylcholine were released during stimulation would suggest that in the absence of eserine the released acetylcholine was not only immediately hydrolysed by the cholinesterase, but that the choline thus formed was at once resynthesized to a 'bound' form of acetylcholine.

In fact, the output of choline actually observed lay midway between these two hypothetical results. There is a transient increase in the output of choline during the first 5 min stimulation, and thereafter there is no evidence of any additional release of choline in spite of continued stimulation. It might be assumed that the choline released during the first 5 min of stimulation is derived from structures in the perfused tissue other than the superior cervical ganglion, and in that case, the second of the hypothetical explanations would be the correct one; namely that, in the absence of eserine, the output of acetylcholine remains consistently high, but that it is immediately hydrolysed and the choline used in the resynthesis of a 'bound' form of acetylcholine. This, if correct, fits in with the hypothesis originally advanced to explain the findings in the presence of eserine, since the available stock of acetylcholine in the ganglion would hardly be depleted during stimulation, and each preganglionic volley, liberating a constant fraction of the stock, would release almost the same total amount of acetylcholine. There would, of course, probably be a limit to this in so far as the enzymic destruction and resynthesis might not be sufficiently rapid to cope with the released acetylcholine during very fast rates of stimulation; in such cases there might still be a fall in the output of acetylcholine even in the absence of eserine.

All the known facts about acetylcholine release from the ganglion can be accounted for by the following tentative hypothesis, which is based on a differentiation of the total acetylcholine in the ganglion into two components, an 'available' stock which can be released by stimulation and a total extractable stock which cannot be released by stimulation but which can be extracted by the usual procedures. Such a differentiation has already been proposed, notably by Abdon & Hammarskjöld (1944). A single preganglionic volley liberates a constant fraction of the stock of 'available' acetylcholine. In the absence of eserine the acetylcholine is immediately hydrolysed and the choline is used in the resynthesis of 'available' acetylcholine. Continuous stimulation is thus accompanied by continuous release of the same amount of acetylcholine per volley (except perhaps at very high rates of stimulation) since the stock of 'available' acetylcholine is little depleted. In the presence of eserine the hydrolysis of the liberated acetylcholine is prevented and consequently resynthesis cannot take place. Continuous stimulation thus leads to a depletion of the stock of 'available' acetylcholine. Replacement of the stock of 'available' acetylcholine is a slow process and occurs at a constant rate of about 4 mμg per min, which corresponds to the rate of liberation of acetylcholine during continuous stimulation after the 'available' stock has been used up. Synthesis of the total extractable acetylcholine in the ganglion is, however, a very rapid process and it is thus very difficult to demonstrate a reduction in the amount of extractable acetylcholine. The synthesized acetylcholine, however, is converted to 'available' acetylcholine only at the slow rate quoted, namely 4 mμg per min. For acetylcholine to become 'available' either a further chemical change or a change in its location may be required.

SUMMARY

1. The output of acetylcholine produced from the perfused eserinized cat's superior cervical ganglion by preganglionic stimulation falls approximately exponentially.

2. The total output of acetylcholine is little influenced by varying the frequency of the preganglionic stimulation between 5 and 100 per sec; thus the output per volley falls more rapidly and to a lower level, the higher the frequency of stimulation.

3. The initial output of acetylcholine per volley is estimated to be of the order of 100 $\mu\mu$g.

4. The output of acetylcholine from a perfused eserinized ganglion in a second period of stimulation after a rest period following an initial period of 40 min stimulation is much reduced unless the rest period is made very long (35 min). This reduction in output is not observed if the initial period of stimulation is carried out in the absence of eserine, either with the circulation intact or with a non-eserinized perfusion.

5. The output of choline from a non-stimulated perfused ganglion in the absence of eserine is initially very high but becomes fairly constant after about 30 min at a level of 20–60 mμg/min.

6. Stimulation increases the output of choline by 20–50 mμg/min for about 5 min but thereafter there is no increase in the amount of choline liberated over the resting level.

7. All the results are compatible with the tentative hypothesis that the amount of acetylcholine liberated from the ganglion by a single preganglionic volley is a constant fraction of the 'available' stock; and that the synthesis of 'available' acetylcholine is a constant, independent of the rate of stimulation, at about 4 mμg/min, the synthesis of total extractable acetylcholine being much more rapid.

On this hypothesis the action of eserine is to prevent the hydrolysis and consequent resynthesis of the liberated acetylcholine, thus depleting the 'available' stock. In the absence of eserine, it is suggested, the 'available' stock is little depleted and the amount of acetylcholine liberated per volley remains almost constant.

REFERENCES

ABDON, N. O. & HAMMARSKJÖLD, S. O. (1944). Is any free acetylcholine preformed in resting muscles or in the heart? *Acta physiol. scand.* **8**, 75–96.

BROWN, G. L. & FELDBERG, W. (1936). The acetylcholine metabolism of a 'sympathetic ganglion'. *J. Physiol.* **88**, 265–283.

FELDBERG, W. & GADDUM, J. H. (1934). The chemical transmitter at synapses in a sympathetic ganglion. *J. Physiol.* **81**, 305–319.

FELDBERG, W. & VARTIAINEN, A. (1934). Further observations on the physiology and pharmacology of a sympathetic ganglion. *J. Physiol.* **83**, 103–128.

FLETCHER, J. P., BEST, C. H. & SOLANDT, O. M. (1935). The distribution of choline. *Biochem. J.* **29**, 2278–2284.

GUGGENHEIM, M. & LOEFFLER, W. (1916). Über das Vorkommen und Schicksal des Cholins im Tierkörper. Eine Methode zum Nachweis kleiner Cholinmengen. *Biochem. Z.* **74**, 208–218.

HOLTON, P. & PERRY, W. L. M. (1951). On the transmitter responsible for antidromic vasodilatation in the rabbit's ear. *J. Physiol.* **114**, 240–251.

KIBJAKOW, A. W. (1933). Über humorale Übertragung der Erregung von einem Neuron auf das Andere. *Pflüg. Arch. ges. Physiol.* **232**, 432–443.

MACINTOSH, F. C. (1938). Liberation of acetylcholine by the perfused superior cervical ganglion. *J. Physiol.* **94**, 155–169.

MACINTOSH, F. C. & PERRY, W. L. M. (1950). Biological estimation of acetylcholine. In *Methods in Medical Research*, **3**, pp. 78–92. Chicago Year Book Publishers.