'PHYSIOLOGICAL AND PHARMACOLOGICAL INVESTIGATIONS ON SYNAPTIC TRANSMISSION IN SYMPATHETIC GANGLIA'

By

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I hereby declare that, with the exception of a few experiments on the action of pentamethonium and hexamethonium iodides (C_5 and C_6) in collaboration with Dr. J. W. Saunders, all of this thesis is my own original work.

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I INTRODUCTION

In vertebrates, outside the spinal column, lies the long sympathetic chain of nerve fibres studded with ganglia whose neurones innervate the glands, heart and smooth muscle. The preganglionic fibres arise from neurones in the spinal cord and leave it in the ventral roots. Thence by way of the white rami they reach the sympathetic trunk and sometimes run up or down several segments of the chain before ending synaptically on ganglion cells. The sympathetic ganglion cells were shown by the histological studies of Cajal (1911), Castro (1932) and others to possess a branching dendrite system which is practically enclosed in a thick capsule, quite unlike the wide and free distribution of the motoneuronal dendrites. The intracapsular dendrites form an open network uniformly distributed around the cells and end in curious shaped swellings either on the capsule itself or amongst the small cells in the capsular sheath called satellite cells by Cajal (1911) and Castro (1932).

There is good experimental evidence that there are no interneurones in the ganglia between the preganglionic fibres and the ganglion cells. Thus Ranson and Billingsley (1918) found that section of the cervical sympathetic, i.e. the preganglionic fibres, produced degeneration of all the fine branching fibres in the ganglion, only the ganglion cells and their axons surviving. Secondly the uniformity of the preganglionic endings and the ganglion cells made it seem very unlikely that interneurones were present (Cajal, 1911 and Castro, 1932; also Ranson and Billingsley, 1918). Since the number of postganglionic fibres far exceeds the number of preganglionic fibres, each preganglionic fibre must innervate several ganglion cells (Langley, 1900). Billingsley and Ranson (1918a) estimated a 1:32 ratio on histological counts though later work indicated a 1:15 ratio (Wolf, 1941).

The following additional evidence also relates to the simple synaptic structure of the ganglion. When nicotine was painted on the superior cervical ganglion (Langley, 1900), transmission through the ganglion was blocked. Similarly section of the preganglionic nerve trunk produced no degeneration beyond the ganglion, i.e. degeneration could affect only the preganglionic trunk and not the internal carotid nerve (Ranson and Billingsley, 1918). Electrical stimulation of the postganglionic nerve produced no activity in the preganglionic nerve - a very good indication, as Brown (1934) points out, for unidirectional conduction through ganglia. The sympathetic ganglion thus provides the simplest example in vertebrates of a synaptic system between two groups of nerve cells, the axons of the preganglionic neurones ending on the ganglion cells without the complication of interneurones.

In the cat there are two types of post ganglionic fibres in the internal carotid nerve, a small group of myelinated fibres and unmyelinated fibres (Billingsley and Ranson, 1918b). On the other hand the postganglionic nerve from the superior cervical ganglia of rabbits contain only unmyelinated nerve fibres (Bishop and Heinbecker, 1932). Occasionally a few sensory fibres traverse the ganglion without synapsing - these usually join the vagus and are not included in the postganglionic trunk. The electrical studies of Bishop and Heinbecker (1932) showed a correlation between threshold of the preganglionic fibres and the function of the ganglion cells they activate. In the cervical sympathetic trunk the low threshold fibres supply the smooth muscle in the iris and the nictitating membrane, the higher threshold fibres being responsible for pilomotor and vasoconstrictor actions. The refractory period for the superior cervical sympathetic ganglia of the rabbit was given as 20 msec by Bishop and Hein-

becker, however Brown (1934) found a figure of only 2 msec for synaptic delay, and a value for refractory period in the order of two msec in the cat superior cervical ganglia.

It is fairly evident that there is normally an asynchronous barrage of activity on to the sympathetic cells because there is experimental evidence that section of the preganglionic or postganglionic cervical sympathetic produces dilitation of the ear blood vessels, reduction in the tone of the nictitating membrane and dilitation of the pupil. Adrian, Bronk and Phillips (1932) found an asynchronous firing of impulses in the cervical sympathetic of anaesthetized rabbits. Since the rate varied with the respirations it was suggested to be the effect of the respiratory centres on the vasomotor centres which was producing a slight alteration in the tone of the blood vessels.

As Bronk (1939) indicated, the most direct method of measuring the manner in which a ganglion cell behaves is to record the activity in different preganglionic fibres converging on a particular ganglion cell and the relationship between this activity and the impulse discharge down the axon. At present the method of recording the ganglionic response, either by the effector organ, or better by the impulses in the postganglionic fibres, remains the only possible way of examining the alteration in activity of ganglion cells. However it is not forgotten that investigation of the ganglionic response to a series of synchronized electrically initiated volleys in the preganglionic fibres may not provide a complete explanation of the effects of drugs on the normal saynchronous discharge of impulses.

The type of transmission to be expected in sympathetic ganglia was formulated in the first instance by Langley and his co-workers who

suggested that the post-synaptic membrane must have receptors for the transmitter substance. Interest in Ach as the probable transmitter was first aroused in 1932 when Kibjakow produced evidence that stimulation of the preganglionic fibres caused the appearance of a ganglionic stimulating substance in the perfusate. Feldberg and Vartiainen (1934b) observed in eserinized perfusates an Ach output on stimulation of the preganglionic fibres but not the postganglionic fibres. Brown and Feldberg (1936b) found little difference between the responses of perfused ganglia and ganglia with their blood supply intact. Addition of potassium chloride in amounts greater than normal could liberate Ach from these ganglia in situ. Their preliminary studies on Ach content of denervated ganglia (Brown and Feldberg, 1936b), were confirmed by MacIntosh's studies on the effect of section of the preganglionic nerve which revealed a 25% decrease in Ach content of ganglion in 24 hours while 72 hours after section only 15% of the Ach was left (MacIntosh, 1938a). Kahlson and MacIntosh (1939) showed the dependence of Ach output on metabolism and the glucose requirements of the ganglion.

Running parallel with the development of the chemical transmitter story were the studies by Eccles (1935, 1936, 1937) and Bronk et al. (1936, 1937, 1938, 1939) on the electrical responses of ganglion cells. Leading from the postganglionic trunk of the superior cervical ganglion of the rabbit or Belgian hare, Eccles showed that the generation of an action potential by the ganglion cells whether stimulated ortho- or antidromically, was followed by a negative and positive afterpotential. The long positive afterpotential with a peak at 100 msec coincided with a period of decreased excitability to antidromic volleys and preganglionic stimuli. However facilitation of the testing volley occurred if the second or testing stimulus was applied to the same preganglionic fibres as the condition-

ing volley. The depression of the ganglion cell during the positive positive afterpotential was illustrated by the complete disappearance during the positive afterpotential of the asynchronous firing of the ganglion cells that was evoked by perfused Ach. The discharge of impulses to the perfused Ach increased as the positive afterpotential decayed (Bronk, 1939).

Preganglionic fibres are known to be capable of following frequencies of up to 300-400/sec. The postganglionic unmyelinated fibres have been shown to conduct impulses satisfactorily up to 150/sec. Ganglion cells are stated (Bishop and Heinbecker, 1932; Bronk and Pumphrey, 1935; Bronk, 1939) to be capable of following only very low frequencies. The failure of response seemed to be a property of the synaptic mechanism. This failure to respond to high frequencies may be due to several factors, anaesthetic, anoxia and temperature. Bishop and Heinbecker's ganglia preparations are unlikely to be at a temperature greater than 30° C and at such a low temperature ganglia are known to fail rapidly (Eccles, 1935). Anaesthetics produce a reduction in the response of ganglion cells as Bishop and Heinbecker commented on the "sluggish behaviour" when urethane was used as the anaesthetic. However Eccles (1935a) ~~Brown (1936)~~ found that there was very little alteration in ganglionic potentials when nembutal was employed as an anaesthetic. The effect of anaesthetics on ganglionic potentials was very carefully investigated by Larrabee and Holaday (1952), and Larrabee and Posternak (1952). No experimental evidence was found to support the theory that anaesthetics primarily affected the oxygen uptake of sympathetic ganglia (Larrabee, Ramos and Bulbring, 1952).

Like all nerve cells especially of warm blooded animals there is a high rate of metabolism and therefore a large requirement for oxygen.

Bronk, Larrabee, Gaylor and Brink (1938) indicated that oxygen lack led to a reduction of the ganglion response whether this response was evoked by the perfusion of Ach or potassium or by the preganglionic impulses. It seems then to be a failure in the excitability of ganglion cells rather than a reduction in the effectiveness of the preganglionic terminals though this could be simultaneously developing. Bronk (1939) reported that conduction of impulses in the fibres traversing the ganglion without synapsing failed at the same time as the transmission across synapses. Later work (~~Bronk~~ Bronk, Larrabee, and Gaylor, 1948) verified this finding of simultaneous failure in transmission across synapses and conduction through ganglia. When high rates of stimulation are employed during anoxia, the development of blockage is greatly accelerated (~~Bronk~~ Bronk, Larrabee, and Gaylor, 1948). The blockage of transmission is almost completely reversible if the ~~asphyxia~~ duration of asphyxia is not greater than two hours (Bronk, 1938; Bronk, Larrabee, Gaylor and Brink, 1938). Even after six hours (Bronk, 1939) 20% of the cells recovered. This remarkable ability to recover from a long period of asphyxia is in marked contrast to the neurones of the central nervous system (Gerard, 1932; Bronk, Larrabee and Davis, 1946) and the p-sympathetic ganglia, which were shown to be unable to recover their excitability after a short exposure to low oxygen pressure (Perry and Talesnik, 1953).

The energy requirements of ganglion cells are not as great as the neurones of the central nervous system but are very much greater than the requirements of peripheral nerves. Larrabee and Bronk (1952) showed little difference in the metabolic requirements of preganglionic and postganglionic fibres or the ganglion cells themselves. Metabolic inhibitors reduce the oxygen uptake of ganglion cells as of any other tissues, however anaesthetics

block conduction across the synapses without having any effect on metabolism as measured by oxygen uptake and glucose utilization.

Certain changes in the characteristic discharge of ganglion cells after prolonged preganglionic tetanus were observed by Bronk (1939) and Larrabee and Bronk (1947). During the post-tetanic period a test volley could elicit a response up to four times normal. It was shown that the potentiation was due to alteration in the preganglionic terminals rather than to some change in post-synaptic characteristics i.e. properties of the ganglion cells. A full description of post-tetanic effects is reserved for a later section.

Further investigations on the effect of drugs on ganglionic transmission have substantiated the theory of Ach as transmitter. Curare in a concentration of 1.0 mg/Kg was found to block transmission across the synapses of the cat's stellate and yet presynaptic impulses still set up a post-synaptic negativity - the synaptic potential (Eccles, 1943). The synaptic potential was 10-15% of the spike discharge, with a time half-decay of 40-60 msec. On repetitive stimulation at frequencies greater than 10/sec the synaptic potentials summed to reach a negative plateau which was maintained throughout the period of stimulation. This plateau level was reached earlier and its height was higher, the faster the stimulus frequency until a maximum was obtained with rates of 120/sec. The negative plateau decreased in two phases, a fast decaying fraction and a slow one. An injection of eserine (1 mg/Kg) greatly prolonged the slow second fraction without a great increase of the duration of the fast decaying phase. This long period of depolarization was readily explained to be due to the presence of the Ach liberated from the preganglionic terminals by stimulation and still unhydrolysed as the enzyme has been inhibited by eserine.

Certain substances like potassium and adrenaline are known to alter the ganglionic response to preganglionic activity. Vogt found an increase in the potassium content of perfusate after stimulation (Vogt, 1936). Brown and Feldberg (1936b) showed an increase in the amount of Ach in the perfusate when potassium chloride was injected. However the sensitivity to potassium was not altered by curare which makes it seem probable that the higher sensitivity of ganglion cells is due to the depolarizing action of the potassium on the post-synaptic membrane. Adrenaline has been postulated to aid transmission in low concentrations (Bulbring and Burn, 1942b; Bulbring, 1944) and block when in high concentrations (Marrazzi, 1939; Marrazzi and Marrazzi, 1947; Bulbring, 1944).

Recently the discovery of new drugs has shown that the blocking action of different substances can be divided into three classes: 1) those that depolarize the post-synaptic membrane thereby producing block; 2) those that have no depolarizing action and yet actively compete with Ach for receptors on the post-synaptic membrane; and 3) those that block the pre-ganglionic terminals. In 1953 Paton and Perry using a direct-coupled amplifier illustrated the difference between the depolarizing action of C_{10} and nicotine and the blocking action without accompanying depolarization by C_5, C_6 and the toxiferins.

Laporte and Lorente de Nó (1950a) reported the appearance of large positive potentials when stimulation was applied to the preganglionic fibres of deeply curarized turtle ganglia. These positivities were interpreted as an anelectrotonus imposed on the ganglion cells by blockage of the impulses invading the preganglionic terminals and it was suggested that this anelectrotonus was actually due in uncurarized ganglia to specific inhibitory fibres. It was in an effort to find whether similar potential changes occurred in mammalian sympathetic ganglia that

initiated the work on these isolated curarized ganglia (R. Eccles, 1952b). The discovery of identical potentials was followed by an attempt to analyse these by pharmacological means.

The pharmacology of mammalian sympathetic ganglia up to 1950, had only been explored in blood circulated or perfused ganglia. The work of Bronk (1939) indicated that ganglion cells were curiously resistant to oxygen lack and could recover their excitability after several hours of anoxia. However until recently isolated ganglia had been used for only one hour after excision (Larrabee, Bronk & Gaylor, 1948). Malcolm (1949), Saunders and Sinclair (1949) and Laporte and Lorente de Nó (1950a,b) had kept excised amphibian and turtle ganglia in good condition for many hours. While this present work was in progress, Brown and Pascoe (1951) reported that isolated mammalian ganglia under favourable conditions could discharge impulses twenty-four hours after excision.

Complete isolation of the ganglion gives two advantages. Firstly, with the isolated ganglion it is possible to use any combination of leads for electrical analysis without the complications of electrical recording which arise from attachment of the ganglion to the animal. Secondly, the ganglion can be subjected to the action of substances in known concentration. Possible disadvantages are that the isolated ganglion would be a deteriorating preparation, and that a long diffusion time is required before there is equilibration with any fluid in which it is immersed. Deterioration has not been serious, because even 36 hours after excision the action potentials have not noticeably changed, and diffusion time for equilibration has been shortened to about 20 minutes by incising the sheath and partly removing it (cf. Rashbass and Rushton, 1949; Feng and Liu, 1949a, 1950).

The rabbit's superior cervical ganglion has the advantage that a relatively long length of post-ganglionic trunk is available for experimental purposes. This advantage is somewhat offset by the occasional presence of double ganglia, already described by Bishop (1936). However it would seem (Bishop, 1936) that the post-ganglionic fibres from the first ganglia join the vagus and were not usually included in the post-ganglionic trunk used. Bishop and Heinbecker (1932) reported the absence of afferent fibres passing uninterruptedly through the ganglion, but only a few low threshold fibres of this type have been observed in some of the present experiments.