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A POSSIBLE ROLE OF AN INHIBITORY SYSTEM IN VIRUS-INFECTED SYMPATHETIC GANGLIA OF THE RAT

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Spontaneous impulses are discharged simultaneously and synchronously in pre- and post-ganglionic nerves of rat sympathetic ganglia infected with pseudo-rabies virus (Dempsher, Larrabee, Bang & Bodian, 1955). The virus-induced activity in the preganglionic nerve is antidromic (in a direction opposite to that occurring normally), whereas in the post-ganglionic nerve the activity is orthodromic or in the normal direction. In order to explain this derangement, the assumption was made that the activity had its origin in the preganglionic nerve endings and was produced by spontaneously released acetylcholine (ACh) acting upon the preganglionic nerve endings made super-irritable by the virus infection (Dempsher & Riker, 1957). In support of this assumption were the following observations. Failure of virus-induced activity to develop in ganglia infected after preganglionic denervation, and the presence of spontaneous impulses in the preganglionic nerve in advanced infections whenever electrical stimulation failed to evoke a post-ganglionic response, supported the view that the site of origin (pace-maker) of the activity was located in the presynaptic nerve. Suppression of spontaneous impulses in the preganglionic nerve whenever that nerve was deprived of its endings, and suppression of activity occurring only in the post-ganglionic nerve by removal of calcium from the bathing solution, were interpreted to mean that the pace-maker was located in the presynaptic nerve endings. The simultaneous and synchronous increase in activity in both nerves produced by physostigmine, and the simultaneous and synchronous decrease in activity in both nerves whenever tubocurarine was applied, supported the view that the virus-induced activity was caused by ACh, and the site of action of ACh was the pace-maker located in the presynaptic nerve endings. In accord with this interpretation were the following observations. Applied ACh produced a simultaneous and synchronous increase in activity in both nerves, thus

mimicking the actions of physostigmine. Physostigmine produced an increase and tubocurarine a decrease in activity discharged only over the preganglionic nerves of ganglia in such advanced stages of infection that electrical stimulation failed to evoke a post-ganglionic response (Dempsher & Riker, 1957).

The purpose of this paper is to report experiments exploring a possibility that the presynaptic nerve endings were superirritable and released ACh because the virus infection interfered with the normal action of an inhibiting system having its origin in the central nervous system (C.N.S.) and exerting its action on the presynaptic nerve terminations. In addition, experiments will be described in which the possibility was explored that adrenaline, noradrenaline, or gamma aminobutyric acid were the chemical mediators released by the inhibiting system.

METHODS

Preparation of the virus-inoculum, described in detail by Tokumaru (1957), was as follows: Monkey kidney cell cultures were inoculated with L strain of pseudo-rabies virus. After a 48 hr incubation period, virus was harvested in the supernatant fluid after centrifuging infected cultures at 3000 rev/min for 5 min. The virus was used either immediately or after storage up to 2 weeks at $-70°$ C. Rat superior cervical ganglia were infected following intra-ocular inoculations of 0·05 ml. of undiluted virus which corresponded to 10^4 tissue culture infectious doses.

Observations of function in excised superior cervical ganglia of rats were carried out in a manner described in detail by Dempsher *et al.* (1955). Briefly, ganglia in desired stages of infection were excised and suspended upon platinum electrodes in a moist chamber containing a bathing solution equilibrated with a mixture of 95% O_2 and 5% CO_2. Impulses in the pre- and post-ganglionic nerves were recorded simultaneously by means of capacity-coupled amplifiers and a dual-beam cathode-ray oscillograph. Brief electrical stimuli were applied to preganglionic nerves in order to test the effects of chemical agents on conduction, and to test the effects of the virus infection upon synaptic transmission.

Observations of function in preganglionic nerves of infected ganglia *in situ* were carried out in the following manner. The carotid sheath was opened along its entire length in the neck region and the preganglionic nerve was carefully dissected free from other tissues. The activity in preganglionic nerve was picked up by platinum electrodes placed on that nerve about 10 mm from the ganglion. Cocaine was applied to the nerves by means of a small wisp of cotton soaked in a 1% solution. The site of application of cocaine on the preganglionic nerve, and that of division of that nerve between C.N.S. and recording electrodes was 12–15 mm from the ganglion. The site of application of cocaine on the preganglionic trunk between ganglion and recording electrodes was 3–5 mm from the ganglion.

The experiments deal with the application to infected ganglia of cocaine, adrenaline, noradrenaline, gamma aminobutyric acid, thiosemicarbazide, and choline 2–6 xylyl ether bromide (TM-10). All concentrations of gamma aminobutyric acid, thiosemicarbazide, TM-10, adrenaline and noradrenaline, applied to excised ganglia, refer to the final concentration of the salt in the bathing solution.

RESULTS

Cocaine. The effects of cocaine applied to intact preganglionic nerves between C.N.S. and recording electrodes (centrally) in eight infected ganglia appeared to be dependent upon the stage of the disease process. In early infections producing no, or very little spontaneous activity in the preganglionic

nerves (Dempsher *et al.* 1955), cocaine application resulted in activity in the preganglionic nerve if initially activity was not present (Fig. 1). Whenever a little activity was already present in the preganglionic nerve in early infections, cocaine application resulted in greatly increased activity. The functional effects produced by cocaine persisted until the solution was washed away. The interval of time between cocaine application and onset of activity in the eight infected ganglia varied from 15 to 60 sec. In late stages of infection, with considerable preganglionic spontaneous activity and little or none in the post-ganglionic nerves (Dempsher *et al.* 1955), cocaine application

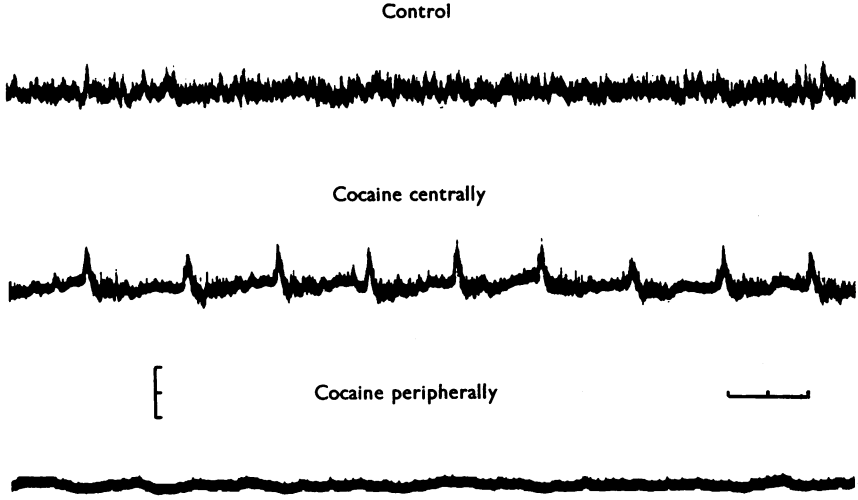

Fig 1. Effects of 1% cocaine applied between C.N.S. and recording electrodes (centrally) and between ganglion and recording electrodes (peripherally) of intact preganglionic nerves of superior cervical ganglia *in situ* in an early phase of pseudo-rabies infection in the rat. Activity appeared 50 sec after it was applied centrally; blockade of activity occurred immediately when applied peripherally. Calibration scales apply to all oscillograms; horizontal time scale, seconds; perpendicular potential scale, 5 μV.

resulted in no, or only a small, increase in that activity (Fig. 2). Cocaine caused immediate blockade of virus-induced activity in the preganglionic nerves whenever applied between ganglia and recording electrodes (peripherally) (Figs. 1, 2); this immediate suppresion of activity is in accord with the assumption that the virus-induced activity originated in the presynaptic nerve endings.

Preganglionic nerve section. The effects of dividing the preganglionic nerve between C.N.S. and recording electrodes in four ganglia in early infections were the same as those following cocaine application to the same area; activity appeared in the preganglionic nerve if initially absent, and increased if initially present. The increase in activity was permanent. Application of cocaine to the cut end of one of these nerves did not alter the pattern of

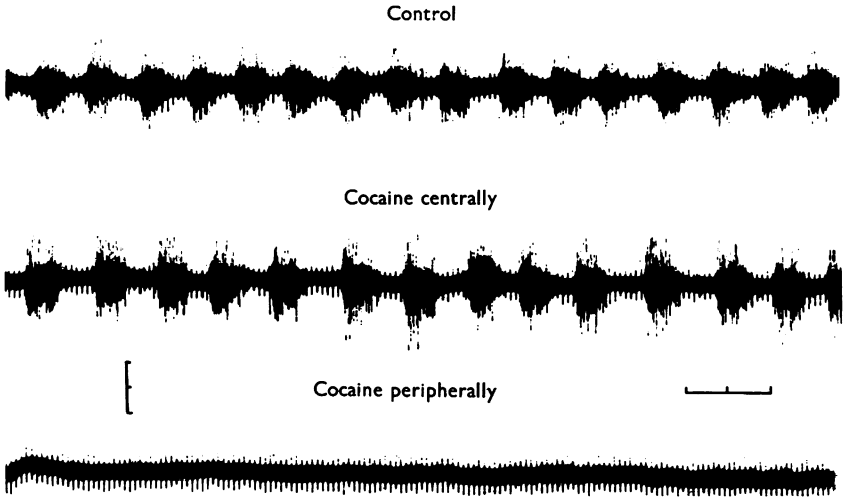

Fig. 2. Effects of 1% cocaine applied between C.N.S. and recording electrodes (centrally) and between ganglion and recording electrodes (peripherally) of intact preganglionic nerves of superior cervical ganglia *in situ* in a late phase of pseudo-rabies infection in the rat. Middle oscillogram was taken 1 min after cocaine application, the bottom oscillogram immediately after cocaine application. Calibration scales apply to all oscillograms; horizontal time scale, seconds; perpendicular potential scale 5 μV. The regular vertical deflexions (best seen in the bottom oscillogram) are the QRS complex of the e.c.g.

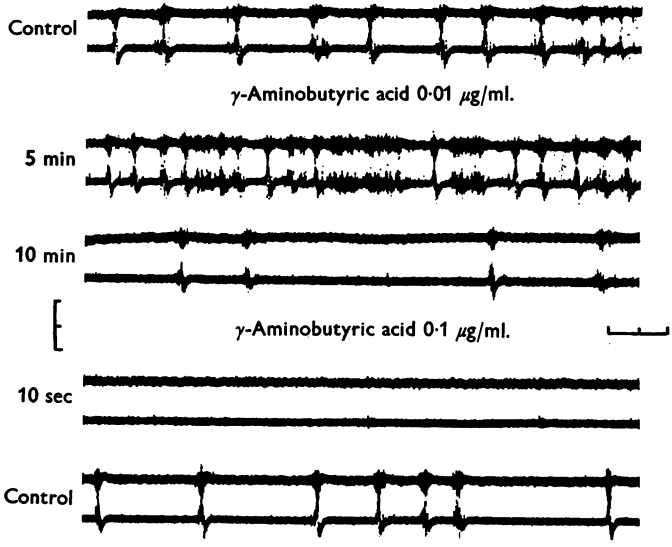

Fig. 3. Effects of gamma aminobutyric acid on spontaneous activity in pre- and post-ganglionic nerves of excised rat superior cervical ganglia infected with pseudo-rabies virus. Upper oscillogram of each pair of action potentials from preganglionic, lower oscillogram from post-ganglionic trunk. Calibration scales below third pair of oscillograms apply to all oscillograms in this figure; horizontal time scale, seconds; perpendicular potential scale, 25 μV.

discharge following nerve section. This confirmed previous conclusions of Dempsher *et al.* (1955) that the injury discharge played no role in the genesis of virus-induced activity.

Gamma aminobutyric acid. In eight excised infected ganglia gamma aminobutyric acid in concentrations of 0·01–10μg/ml. produced initially a simultaneous and synchronous increase, then a simultaneous and synchronous

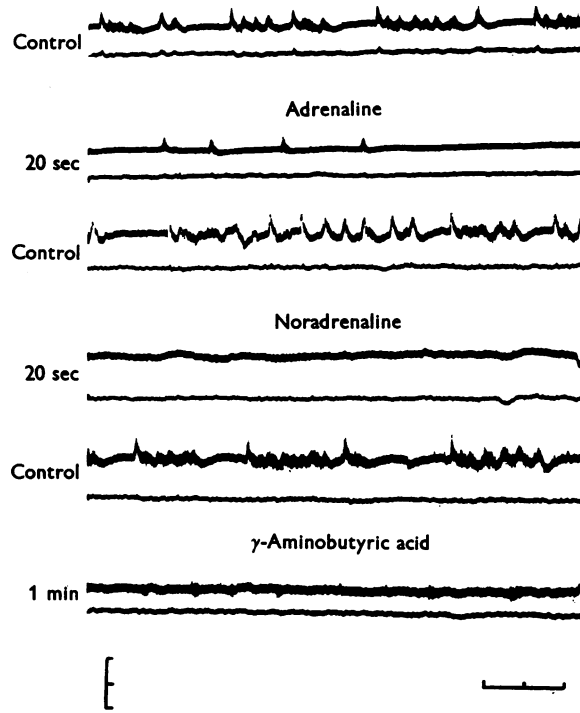

Fig. 4. Effects of adrenaline 1 μg/ml., noradrenaline 10μg/ml., gamma aminobutyric acid 1 μg/ml., on spontaneous activity in the preganglionic nerves of excised superior cervical ganglia of rats in a late phase of pseudo-rabies infection. Upper oscillogram of each pair of action potentials from preganglionic, lower oscillogram from post-ganglionic trunk. Calibration scales below bottom pair of oscillograms apply to all oscillograms in this figure; horizontal time scale, seconds; perpendicular potential scale, 25 μV, applies to action potentials in the upper oscillogram of each pair.

decrease in activity in both nerves (Fig. 3). In late infections, whenever no activity was discharged in the post-ganglionic nerves, and electrical stimulation of the preganglionic nerves failed to evoke post-ganglionic responses, gamma aminobutyric acid caused a decrease in preganglionic activity (Fig. 4). Preganglionic action potentials evoked by electrical stimulation were not affected by gamma aminobutyric acid.

Adrenaline and noradrenaline. Whenever adrenaline 1–10 μg/ml. or noradrenaline 10–100 μg/ml. were applied to excised infected ganglia, the results

obtained were the same as those produced by gamma aminobutyric acid: an initial simultaneous and synchronous increase in activity in both nerves followed by a decrease. In late infections, in which no activity was discharged in the post-ganglionic nerves and in which electrical stimulation failed to evoke post-ganglionic responses, adrenaline and noradrenaline abolished activity in the preganglionic nerves (Fig. 4). Preganglionic action potentials evoked by electrical stimulation were not affected by adrenaline or noradrenaline.

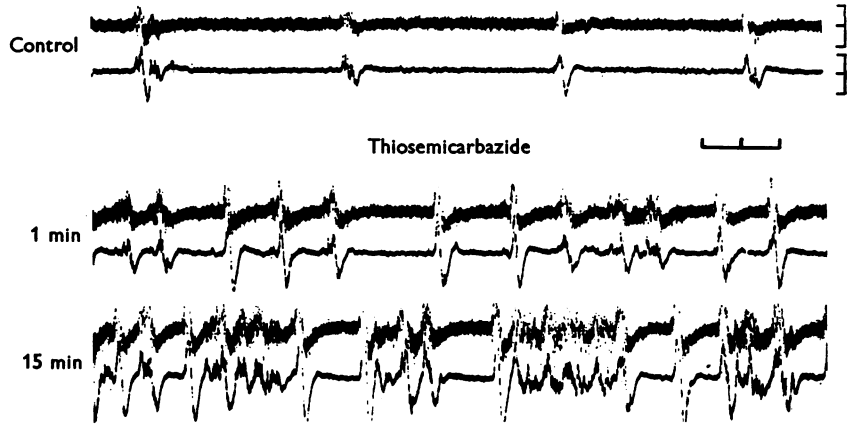

Fig. 5. Effects of thiosemicarbazide 0·01 μg/ml. on spontaneous activity in pre- and post-ganglionic nerves of excised sympathetic ganglia of rats infected with pseudo-rabies virus. Upper oscillogram of each pair of action potentials from preganglionic, lower oscillogram from post-ganglionic trunk. Upper perpendicular potential scale applies to the upper oscillogram of each pair of action potentials and shows 5 μV. Lower perpendicular potential scale applies to the lower oscillogram of each pair of action potentials and shows 20 μV; the horizontal time scale shows seconds.

Thiosemicarbazide. In eight excised infected ganglia thiosemicarbazide in concentrations of 0·01–100 μg/ml. produced a simultaneous and synchronous increase in spontaneous activity in both nerves (Fig. 5).

TM-10. In concentrations of 1–10 μg/ml. TM-10 produced effects similar to those produced by thiosemicarbazide, a simultaneous and synchronous increase in virus-induced activity in both nerves.

DISCUSSION

It has been concluded that spontaneous activity, discharged antidromically in preganglionic nerves and orthodromically in post-ganglionic nerves of rat sympathetic ganglia infected with pseudo-rabies virus, originated in the preganglionic nerve endings and was caused by ACh (Dempsher *et al.* 1955; Dempsher & Riker, 1957). The present observations explore the possibility that ACh was released because the virus infection had interfered with the normal action of an inhibiting system having its origin in the C.N.S. and

exerting its effects upon the excitatory mechanism in the presynaptic nerve endings. In support of such a view were the appearance of activity when initially absent, and the increase in activity already present in preganglionic nerves of infected ganglia following cocaine application or nerve section between C.N.S. and preganglionic recording electrodes. It is presumed the above procedures blocked inhibitory fibres which originate in the C.N.S. and exert their effects upon the presynaptic nerve endings, so as to allow the virus-induced activity originating in the presynaptic nerve endings to appear or increase. An alternative explanation of these observations is that the virus-induced activity appeared to be suppressed simply because some of the nerve fibres were already occupied by orthodromic excitatory impulses originating in the C.N.S. Blockade of such orthodromic excitatory impulses from the C.N.S. with cocaine or nerve section would thus permit the appearance or increase in virus-induced antidromic activity. However, if this were the case, then the apparent suppression of virus-induced activity by simple occupation of nerve fibres by excitatory impulses should be equally effective in late stages of the infection. This was not the case, since, as the disease progressed, the activity increased with little or no effect from cocaine or nerve section. Furthermore, the relatively long quiet period of 15–60 sec following cocaine application and onset or increase in virus-induced activity in early infections suggested inactivation of an inhibitory substance suppressing the activity rather than recovery of nerve conduction from a refractory period. The long quiet period did not appear to be due to a slow onset of action of cocaine, since immediate suppression of activity occurred whenever it was applied between ganglion and recording electrodes.

At the present time it is not known how an inhibiting system could exert its effects at presynaptic nerve endings nor how the virus infection could interfere with it. The experimental observations suggest the possibility that the actions of the inhibiting system were produced by chemical mediators, and that interference in the formation of these substances resulted in an imbalance between excitatory and inhibitory systems which was manifested by the appearance of spontaneous activity. The simultaneous and synchronous increase in activity in both nerves produced by thiosemicarbazide and TM-10 suggested the possibility that the inhibiting mediators may include gamma aminobutyric acid and one or more of the catechol amines. Killam & Bain (1957) concluded that the thiosemicarbazide-induced seizures in rats were due to decreased levels of gamma aminobutyric acid resulting from inhibition of glutamic acid decarboxylase, the enzyme catalysing the formation of gamma aminobutyric acid from glutamic acid. In addition, Coupland & Exley (1957) suggested that the decreased levels of adrenaline and noradrenaline in suprarenal glands of rats treated with TM-10 was caused by decreased synthesis of catechol amines. Also in accord with our suggestion

were the simultaneous and synchronous decrease in activity in both nerves whenever gamma aminobutyric acid, adrenaline, and noradrenaline were added to infected ganglia. Since the formation of these chemical agents from their precursors is believed to be dependent at one stage upon a process of decarboxylation (Roberts & Frankel, 1951; Udenfriend & Wyngaarden, 1956), it is conceivable that the virus infection interfered with this process, thus upsetting the balance between excitatory and inhibitory systems.

The observation that simultaneous and synchronous increase in activity in both nerves always preceded the decrease whenever gamma aminobutyric acid, adrenaline, or noradrenaline were applied to infected ganglia can probably be explained in the following manner. It is not unlikely that the resting membrane potentials of the presynaptic nerve endings of infected ganglia were in a partially depolarized state. Since there is some evidence that blockade by gamma aminobutyric acid and adrenaline is accompanied by a membrane potential in the polarized state (Lundberg, 1952; Edwards & Kuffler, 1957), it is possible that these substances moved the partially depolarized membrane potential through a value optimal for maximum excitability before it was fixed to cause total blockade. The excitatory actions of adrenaline and noradrenaline on cat ganglia were observed by previous investigators (Bülbring & Burn, 1942; Bülbring, 1944; Trendelenburg, 1956).

The following working hypothesis as a concept of functional organization in rat superior cervical ganglion is proposed. Impulses are conducted to presynaptic nerve endings from the C.N.S. over two systems of nerve fibres, an excitatory and an inhibitory system. The resultant of events taking place in the presynaptic nerve endings is then transmitted to the post-synaptic nerve cells. The events occurring in the presynaptic nerve endings would include the synthesis, release, actions and inactivation of the chemical mediator of excitation, ACh, and of chemical mediator(s) of inhibition, possible candidates being gamma aminobutyric acid, adrenaline, and noradrenaline. Dempsher & Riker (1957) concluded that the site of action of ACh was at the presynaptic nerve endings. The simultaneous and synchronous effects in both pre- and post-ganglionic nerves whenever gamma aminobutyric acid, adrenaline, and noradrenaline were applied indicate that the actions of the inhibiting substances would also be on the pace-maker located in the presynaptic nerve endings. In accord with this view were the simultaneous and synchronous effects on both nerves produced by thiosemicarbazide and TM-10, agents believed to interfere with the formation of the inhibiting substance. Furthermore, the decrease in activity produced by gamma aminobutyric acid, adrenaline, and noradrenaline whenever applied to ganglia in late infections in which activity is present only in the preganglionic nerves, was shown to be due to their effects upon the presynaptic nerve endings.

The view that rat superior cervical ganglia may possess an inhibitory

system releasing adrenaline, noradrenaline, and gamma aminobutyric acid has some support from the work of other investigators who found block of synaptic transmission by adrenaline and noradrenaline in autonomic ganglia (Marazzi, 1939; Bülbring, 1944; Lorente de Nó & Laporte, 1950; Trendelenburg, 1956). Bülbring (1944) has further shown that an adrenaline-like substance appears in the effluent of the perfused superior cervical ganglion on preganglionic stimulation. Block of synaptic transmission by gamma aminobutyric acid has also been observed in the stellate but not in the superior cervical ganglion of cats (Bazemore, Elliott & Florey, 1956). In the present experiments, the superior cervical ganglion of the rats, however, was found to be extremely sensitive to the blocking action of this amino acid.

SUMMARY

1. Spontaneous impulses arising in rat superior cervical ganglia following infection with pseudo-rabies were studied *in vivo* and *in vitro* in these ganglia with standard electrophysiological techniques.

2. In early infections no, or little, activity was discharged in the intact preganglionic nerves *in vivo*, whereas in late infections a considerable amount of virus-induced activity was discharged in the intact preganglionic nerves.

3. Blockade of conduction in preganglionic nerves by cocaine or nerve section between C.N.S. and recording electrodes of ganglia in early infections was followed by the appearance of virus-induced activity in the preganglionic nerve if initially absent, and increase in activity if initially present. A similar procedure carried out on ganglia in late infections was followed by no, or very little, increase in the considerable amount of virus-induced activity already present in the preganglionic nerves.

4. Thiosemicarbazide and TM-10 produced a simultaneous and synchronous increase in activity in both pre- and post-ganglionic nerves of infected ganglia.

5. In early infections, gamma aminobutyric acid, adrenaline and noradrenaline caused initially a simultaneous and synchronous increase followed by a decrease in activity in both pre- and post-ganglionic nerves. In ganglia in such advanced stages of infection that no activity was present in the post-ganglionic nerve, gamma aminobutyric acid, adrenaline and noradrenaline caused a decrease in virus-induced activity in the preganglionic nerve.

6. It is suggested that spontaneous impulses may arise in rat superior cervical ganglia following infection with pseudo-rabies virus because the virus infection has interfered with the normal action of an inhibitory system originating in the C.N.S. and exerting its actions upon the presynaptic nerve endings. It is also suggested that adrenaline, noradrenaline and gamma aminobutyric acid are possible substances released by this system.

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