THE EFFECT OF SUBSTANCE P ON THE SUPERIOR CERVICAL GANGLION OF THE CAT

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Intra-arterial injections of substance P in doses from 10 to 30 units potentiated the response of the nictitating membrane to submaximal stimulation of the preganglionic sympathetic nerve, while higher doses (30 to 100 units) usually depressed the response. The stimulating action of acetylcholine on the superior cervical ganglion (as judged by the response of the nictitating membrane) was also potentiated by substance P. The responses of the nictitating membrane to adrenaline, noradrenaline and tyramine were potentiated by substance P as well. It is concluded that the potentiating effect of substance P on the response of the nictitating membrane to submaximal stimulation of the preganglionic sympathetic nerve is probably due to sensitization of acetylcholine receptors in the postsynaptic neurone. The present experiments do not explain how substance P potentiated the response to sympathomimetic amines.

It has been shown that substance P may restore peristalsis when injected into the lumen of the isolated guinea-pig ileum in which the peristaltic reflex has been abolished by fatigue, by external or internal application of 5-hydroxytryptamine, or by lowering the temperature of the bath (Belesin and Varagić, 1958). Substance P, when acting on the outside of the isolated guinea-pig ileum, blocks the peristaltic reflex (Belesin and Varagić, 1959). In previous work some evidence was obtained that this block of peristalsis might be at least partly produced by the action of substance P on the intestinal ganglia. On the other hand, Lechner and Lembeck (1958) recorded the electroencephalogram of the cortex and of the hippocampus in rabbits after an injection of 30 to 100 units of substance P into the carotid artery and found that there was a decrease in amplitude and an increase in frequency which was most pronounced in 1 to 2 min. It was therefore of interest to investigate the effect of substance P on autonomic ganglia.

METHODS

Cats of both sexes, weighing 1.5 to 3 kg., were used. After inducing anaesthesia with ether, 80 mg./kg. of chloralose was injected intravenously. Intra-arterial injections were made into the central end of the lingual artery while occluding the external carotid artery. Thus the injected substance was diverted towards the superior cervical ganglion.

The contractions of the nictitating membrane were recorded with an isotonic lever fitted with a frontal

writing point magnifying the movements of the membrane ten times. The cervical sympathetic chain was divided and, when stimulated electrically, its peripheral end was placed on shielded electrodes and covered with liquid paraffin. For stimulation an electronic stimulator delivering square wave pulses was used. The pulses had a duration of 0.8 msec. and a frequency between 4 and 15 per sec.

In some experiments the intra-arterial injections were made without occluding the external carotid artery, thus allowing the injected substance to act directly on the nictitating membrane.

Substance P was extracted and purified according to the method described by Zetler (1956).

The following substances were used: substance P, acetylcholine hydrochloride, adrenaline hydrochloride, noradrenaline bitartrate and tyramine hydrochloride. With the exception of substance P all doses are expressed in terms of the salts.

RESULTS

Substance P and Sympathetic Nervous Stimulation. — The intra-arterial injection of substance P in doses from 10 to 100 units changed the response of the nictitating membrane to submaximal stimulation of the preganglionic sympathetic nerve. With the stimulation in periods of 5 sec. every minute, a series of contractions of the nictitating membrane was recorded as shown in Fig. 1. At C the external carotid artery was occluded, and an intra-arterial injection of 18 units of substance P increased the response of the nictitating membrane to the preganglionic stimulation. The external carotid artery was

opened (at O) 10 min. after the injection of substance P, thus allowing the substance to reach the nictitating membrane in a high concentration. The response to preganglionic stimulation was now reduced and 8 min. later was completely abolished. The complete block lasted 15 min. after which the response gradually returned to normal. The amount of substance P which was injected in this experiment did not itself stimulate the ganglion and caused no contraction of the nictitating membrane in the absence of preganglionic stimulation. This type of response was obtained in five out of eleven experiments.

The response of the nictitating membrane to stimulation of the postganglionic sympathetic nerve did not change significantly after intra-arterial injection of substance P. A typical result is shown in Fig. 2. The response of the nictitating membrane to preganglionic stimulation was potentiated immediately after injection of substance P. On the other hand, the response to postganglionic stimulation was unchanged. At O the external carotid artery was opened. After 10 min. the response to preganglionic stimulation was still potentiated but gradually declined, whereas the response to postganglionic stimulation was potentiated, as shown on the right-hand side of the figure.

In some experiments no initial increase in the response to preganglionic stimulation was observed. In these experiments the intra-arterial injection of substance P in doses from 30 to 100 units caused only a gradual decline in the response which sometimes was followed by a complete block. Such a result is shown in Fig. 3 (here the block of the response lasted for more than 45 min.). In four experiments a delayed potentiation of the effect of preganglionic sympathetic stimulation was observed. This effect was observed 30 to 120 min. after injection of substance P.

Substance P and Acetylcholine.—It has been shown previously that some substances may potentiate the response of the superior cervical ganglion to acetylcholine. Thus Konzett (1952; cited by Trendelenburg, 1956) found that histamine potentiated the response of the perfused superior cervical ganglion to acetylcholine. Trendelenburg (1956) confirmed this and found that both 5-hydroxytryptamine and pilocarpine shared this action. In the present experiments substance P also poten-

FIG. 1.—Cat, chloralose. Contractions of the nictitating membrane. At dots: submaximal preganglionic stimulation of the cervical sympathetic nerve (5 pulses/sec., 0.8 msec., 7 mA) for 5 sec. every min. At C, the external carotid artery was occluded. At P, 18 units of substance P was injected into the lingual artery. At O, the external carotid artery was opened. At X, the kymograph was stopped for 15 min. Time, 1 min.

FIG. 2.—Cat, chloralose. Contractions of the nictitating membrane. The external carotid artery was occluded. At every single dot: submaximal preganglionic stimulation of the cervical sympathetic nerve (5 pulses/sec., 0.8 msec., 2 mA) for 10 sec. every 90 sec. At every two dots: submaximal postganglionic stimulation of the cervical sympathetic nerve. At O, the external carotid artery was opened. At P, 18 units of substance P was injected into the lingual artery. Time, 1 min.

tiated the response of the nictitating membrane to acetylcholine. A typical result is shown in Fig. 4. The intra-arterial injection of $30\text{ }\mu\text{g}$. of acetylcholine while the external carotid artery was occluded caused a contraction of the nictitating membrane due to a direct effect on the ganglion cells. These contractions of the nictitating membrane were increased after intra-arterial injection of 100 units of substance P. This effect was clear 12 min. after injection of substance P and it was regularly observed.

FIG. 3.—Cat, chloralose. Contractions of the nictitating membrane. At every single dot: submaximal preganglionic stimulation of the cervical sympathetic nerve (4 pulses/sec., 0.8 msec., 1.2 mA) for 10 sec. every 90 sec. At every two dots: submaximal postganglionic stimulation of the cervical sympathetic nerve (4 pulses/sec., 0.8 msec., 0.1 V.) for 10 sec. every 90 sec. At C the external carotid artery was occluded. At P, 100 units of substance P was injected into the lingual artery. B was taken 20 min. after the injection of substance P. Time, 1 min.

FIG. 4.—Cat, chloralose. Contractions of the nictitating membrane. At dots, $30\text{ }\mu\text{g}$. of acetylcholine was injected into the lingual artery while the external carotid artery was occluded. At O, the same dose of acetylcholine was given while the external carotid artery was patent. At P, 100 units of substance P was injected into the lingual artery. Time, 1 min.

Substance P and Sympathomimetic Amines.—The effect of substance P on the response of the nictitating membrane to sympathomimetic amines was examined while the external carotid artery was patent, thus allowing the sympathomimetic drugs to act directly on the nictitating membrane. In the majority of the experiments it was found that the intra-arterial injection of substance P potentiated the response of the nictitating membrane to noradrenaline, adrenaline and tyramine. Fig. 5 shows such a result in which the response to noradrenaline was potentiated. This effect did not appear immediately after injection of substance P, but after an interval usually of 15 to 20 min.

FIG. 5.—Cat, chloralose. Contractions of the nictitating membrane produced by intra-arterial injections of $20\text{ }\mu\text{g}$. noradrenaline at the dots (the external carotid artery open). A, control. B, 20 min. after injection of 18 units of substance P into the lingual artery while the external carotid artery was open. Time, 1 min.

DISCUSSION

These experiments show that ganglionic transmission in the superior cervical ganglion of the cat may be potentiated or depressed by substance P. The potentiating effect was usually observed after small doses of substance P (10 to 30 units) and depression was usually produced by higher doses (100 units). It is known from the work of Trendelenburg (1956) that a variety of chemically unrelated substances may potentiate or depress ganglionic transmission. These substances can be divided into three groups: the nicotine-like substances (choline esters and nicotine), the sympathomimetic amines (adrenaline and noradrenaline) and a third group of drugs (including histamine, 5-hydroxytryptamine

and pilocarpine). Although all these substances potentiate sympathetic synaptic transmission, depression was obtained only with drugs of the first two groups. Substance P is known to be a polypeptide and hence chemically unrelated to any of these substances, but it can alter synaptic transmission in the sympathetic ganglion.

Substance P was also found to potentiate the response of the ganglion cells to intra-arterial injections of acetylcholine. This effect may be explained by the sensitization of acetylcholine receptors in the postsynaptic neurone. On the other hand, depression by substance P may be due to inhibition of the acetylcholine receptors or to non-specific actions of impurities in the substance P itself.

It is very difficult to explain the mechanism by which substance P potentiates the response of the

nictitating membrane to the sympathomimetic amines which were used in the present experiments. The potentiating effect of substance P on the response of the nictitating membrane to postganglionic stimulation, which was occasionally seen, is in accordance with the finding that the response to noradrenaline was also potentiated.

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