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EFFECT OF HISTAMINE ON GANGLIONIC TRANSMISSION

BY

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Histamine has been shown to depress transmission through the perfused superior cervical ganglion of the cat when doses of 150 μg. or more were administered. The intensity of the ganglionic block was related to the dose of histamine employed. In one-third of the experiments, a slow contracture of the nictitating membrane occurred after histamine had been injected; the contracture lasted up to 10 min., and subsequent injections of histamine gave rise to progressively smaller responses. The blocking action of histamine was evident in all experiments and was the most prominent feature observed. Histamine in a sub-depressant dose enhanced the action of the competitive blocking agents tetraethylammonium and hexamethonium, and also the depolarizing blocking agents tetramethylammonium and nicotine. The possible physiological rôle of histamine in the autonomic nervous system is discussed.

Although the presence of histamine in nervous tissue has been reported by a number of authors, its physiological significance remains obscure. Harris, Jacobsohn, and Kahlson (1952) demonstrated the presence of histamine in considerable quantities in the region of the hypothalamus, and von Euler and Astrom (1948), von Euler (1949), and Werle and Weicken (1949) showed that certain peripheral nerve fibres, particularly the sympathetic postganglionic fibres, were rich in histamine. The release of histamine from certain peripheral nerves on electrical stimulation was reported by Kwiatkowski (1943); and Gertner (1955) has shown that the histamine liberators 48/80 and propamidine depress transmission through the perfused superior cervical ganglion of the cat.

The effects of exogenous histamine on transmission in the nervous system would appear to require further study since divergent results have appeared in the literature. Feldberg and Vartianen (1935) were unable to demonstrate any action of histamine on transmission through the sympathetic ganglion, whereas Burn and Trendelenburg (1954), Trendelenburg (1954, 1955), and Konzett (1952) described a stimulant action of histamine. The present investigation was therefore undertaken in an attempt to establish the actions of histamine on transmission through the sympathetic ganglion and also to ascertain its effects on the actions of ganglion blocking agents.

METHOD AND MATERIALS

Cats of either sex weighing 2 to 3.5 kg. were anaesthetized with pentobarbitone sodium 30 mg./kg. The superior cervical ganglion was then isolated and perfused *in vivo* according to the method of Feldberg and Gaddum (1934) and with the later modification of MacIntosh, as reported by Perry (1953). The perfusion fluid was Locke solution made up as follows: NaCl, 9.2 g.; KCl, 0.42 g.; $CaCl_2$, 0.24 g.; $NaHCO_3$, 0.15 g.; glucose, 1 g./l. of solution. 25 cats were employed.

The nictitating membrane was connected to a frontal writing lever and the response recorded on a smoked paper. The preganglionic cervical sympathetic trunk was stimulated for 10 sec. in each minute by rectangular pulses from a Grass stimulator at 10/sec., of 0.5 m.sec. duration and supramaximal voltage usually 10 V.

The drugs used were dissolved in Locke solution and injected into the perfusion stream in a volume never exceeding 0.3 ml. Histamine was used as the acid phosphate unless otherwise indicated and the doses are quoted as histamine base. The doses of all other drugs are given in terms of their salts.

RESULTS

Effect of Histamine on Ganglionic Transmission

Graded doses of histamine were injected into the perfusion stream. Quantities of histamine from 1 to 100 μg. produced little noticeable effect, whereas larger amounts (100 to 150 μg.) produced a slight depression of transmission. With doses of 300 μg. of histamine, a greater degree of

depression was evident in all the preparations tested and doses of 600 μg. produced a profound inhibition of ganglionic transmission. In two ganglia, there was a complete block of transmission with 600 μg. of histamine lasting for 10 min. Recovery was complete within a period of 20 min. Fig. 1 illustrates the effects of graded doses of histamine.

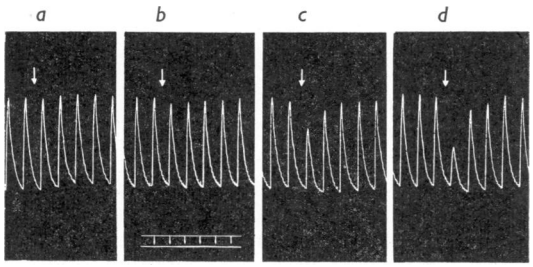

FIG. 1.—Graded responses to graded doses of histamine. Cat 2.5 kg., pentobarbitone anaesthesia, perfused superior cervical ganglion preparation. Contractions of the nictitating membrane in response to electrical stimulation of the preganglionic cervical sympathetic trunk for 10 sec. every min. Injections of histamine into the ganglion perfusion fluid at the arrows (*a*) 75 μg.; (*b*) 150 μg; (*c*) 300 μg., and (*d*) 600 μg. Time, 1 min.

The possibility that this block of transmission was due to the action of the phosphate anion of the histamine molecule was then studied. Histamine dihydrochloride containing the same proportion of base as the phosphate salt produced identical degrees of ganglionic block. This indicated that the depression of transmission was due to the histamine *per se* and not to the anion portion of the molecule. Since the acid phosphate of histamine has a *p*H of 4.2, a control was carried out by preparing a buffer solution of M/15 Na_2HPO_4 and NaH_2PO_4 of identical *p*H and its effect studied on transmission. When 0.2 ml. of this buffered solution was injected into the perfusion stream, no noticeable effect on transmission was observed. It was thus evident that histamine itself, in the doses employed, depressed ganglionic transmission.

In one-third of the experiments it was noted that the first injection of histamine (usually 150 μg.) produced a slow contracture of the nictitating membrane, often sustained for up to 10 min. This occurred in the absence of electrical stimulation of the ganglion (Fig. 2). When the identical dose of histamine was given after recovery, the stimulant effect of the second dose was greatly diminished. A third injection given after recovery produced a still smaller contraction of the nictitating membrane. Both the chloride and the phosphate salts of histamine also showed this tachyphylactic stimulant effect, which is thus clearly due to histamine itself.

This slow contracting action of histamine was completely independent of the other and much more prominent action of histamine, namely that of depressing transmission. Histamine depressed transmission in all the preparations tested and there was no tachyphylaxis in respect of this depression.

Effect of Histamine in the Presence of Ganglion Blocking Agents

Doses of ganglion blocking agents were chosen, which, when injected into the perfusion stream, produced about 30% to 50% depression of transmission. This dose was given three times to determine whether potentiation occurred on repeated injections of the blocking agent. A test dose of histamine was selected which had negligible effects on transmission. The ganglion blocking agent was injected into the perfusion stream and followed within a minute by the test dose of histamine. Two representative competitive blocking agents, tetraethylammonium and hexamethonium, and two depolarizing blocking agents, tetramethylammonium and nicotine, were employed.

Hexamethonium Chloride

Usually doses of 10 μg. of hexamethonium produced approximately a 50% block of transmission. When the test dose of 150 μg. of histamine was injected immediately after the

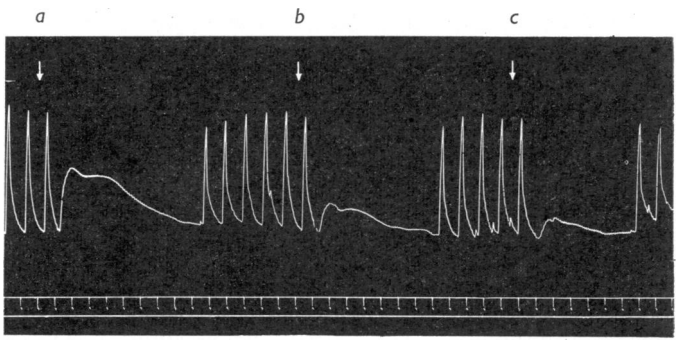

FIG. 2.—Slow contraction produced by histamine. Cat 3.0 kg., pentobarbitone anaesthesia, perfused superior cervical ganglion preparation. Contractions of the nictitating membrane to electrical stimulation of the preganglionic cervical sympathetic trunk for 10 sec. every min. Injections of 150 μg. of histamine into the perfusion fluid at (*a*), (*b*), and (*c*). Electrical stimulation was discontinued 1 min. after the injection of histamine and started again when recovery was complete. Time, 1 min.

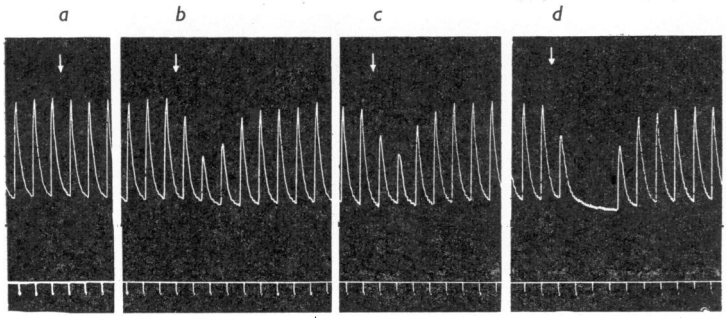

FIG. 3.—Enhancement of the action of hexamethonium by histamine. Cat 2.8 kg., pentobarbitone anaesthesia, perfused superior cervical ganglion preparation. Contractions of the nictitating membrane to electrical stimulation of the cervical sympathetic trunk for 10 sec. every min. At (*a*) 150 μg. of histamine; at (*b*) and (*c*) 10 μg. of hexamethonium chloride; at (*d*) 10 μg. of hexamethonium followed immediately by 150 μg. of histamine. Complete ganglion block arose. Time, 1 min.

third dose of hexamethonium, the degree of depression of transmission through the ganglion was increased both in intensity and duration (Fig. 3). This potentiation by histamine of the inhibiting action of hexamethonium could be repeated after re-establishment of normal transmission through the ganglion.

Tetraethylammonium Chloride

Tetraethylammonium chloride in a dose of about 100 μg. produced about a 50% block of transmission through the ganglion. The procedure as outlined for hexamethonium was followed, and similar results obtained. The response of the ganglion to the tetraethylammonium was greatly enhanced by histamine and this could be repeated on re-establishment of normal transmission 10 to 15 min. following the last injection of histamine.

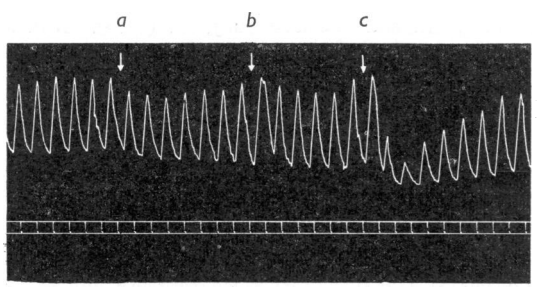

FIG. 4.—Enhancement of the depressant action of tetramethylammonium by histamine. Cat 3.0 kg., pentobarbitone anaesthesia, perfused superior cervical ganglion preparation. Contractions of the nictitating membrane to electrical stimulation of the cervical sympathetic trunk for 10 sec. every min. Injections into the fluid perfusing the ganglion at (*a*) 150 μg. of histamine; at (*b*) 10 μg. of tetramethylammonium bromide; at (*c*) 10 μg. of tetramethylammonium bromide followed immediately by 150 μg. of histamine. Time, 1 min.

Tetramethylammonium Bromide.—A dose of tetramethylammonium bromide, usually 10 μg., was chosen which initially stimulated the ganglion cells and then slightly depressed transmission. When histamine was given after tetramethylammonium, a greatly enhanced depression of transmission was observed (Fig. 4). In all experiments only the post-stimulatory depression phase of the action of tetramethylammonium was potentiated and not the initial stimulant phase. In general, these results could be repeated after complete recovery (10 to 15 min.) with no diminution of effect.

Nicotine

A dose of 5 μg. of nicotine hydrogen tartrate usually stimulated the ganglion cells initially but subsequently depressed transmission to approximately 50% of the original value. After the injection of nicotine, histamine caused considerable enhancement of the depression of transmission (Fig. 5), but only the post-stimulatory depression of transmission was potentiated and not the initial stimulant phase. An injection of 5 μg. nicotine given when recovery was complete (18 min. later) showed that the potentiating action of histamine had entirely disappeared.

DISCUSSION

It is clear from the experiments reported here that histamine *per se* has the ability to depress transmission when injected into the perfused superior cervical ganglion of the cat. This was a distinct pharmacological effect as is evident from the dose/response relationships. The reports in the literature concerning the lack of activity of histamine on the ganglion may be due to its use in insufficient doses.

The stimulating action of histamine on ganglia, which has been reported by other investigators, may be related to the slow contraction described in this paper. This phenomenon, which exhibits tachyphylaxis, is of considerable interest. No explanation as to why it occurred in only one-third of the experiments readily presents itself. The possibility exists that, in a small number of ganglia, some sympathomimetic amine may be released by histamine from chromaffin tissue present in the ganglion and that this may slowly diffuse out and produce the contraction

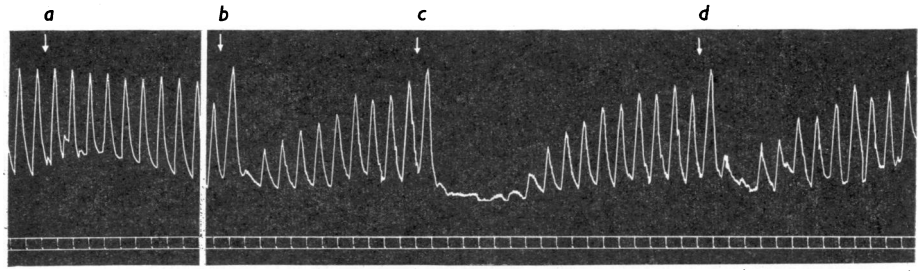

FIG. 5.—Enhancement of the depressant action of nicotine by histamine. Cat 2.9 kg., pentobarbitone anaesthesia, perfused superior cervical ganglion preparation. Contractions of the nictitating membrane to electrical stimulation of the cervical sympathetic trunk for 10 sec. every min. At (*a*) 150 μg. of histamine was injected; (*b*) 5 μg. of nicotine hydrogen tartrate; (*c*) 5 μg. of nicotine hydrogen tartrate followed immediately by 150 μg. of histamine; and (*d*) 5 μg. of nicotine hydrogen tartrate. Time, 1 min.

of the nictitating membrane. A point in favour of such a hypothesis is that it is a tachyphylactic phenomenon suggesting that the substance responsible may become exhausted. The fact that this slow contraction is observed would explain why histamine has been reported by some to exert a stimulant action on ganglia, masking the more important and prominent action of histamine in depressing transmission.

The doses of histamine injected into the ganglion may appear to be somewhat larger than ordinarily required to obtain a physiological response such as that on the cardiovascular system. It is important, however, to point out that, although this preparation remains *in situ*, it is isolated from the normal metabolites of the body as well as from the blood stream. In addition, and possibly of greater importance, is the lack of any substantial evidence on the ability of nervous tissue to metabolize histamine, hence it cannot be concluded justifiably at this stage that the doses employed in our experiments were excessive.

At present it is not possible to claim any physiological rôle for histamine in autonomic transmission, but it is of interest that there may be an analogy between histamine and acetylcholine in this situation. It should be recalled that the dose of acetylcholine required to stimulate the ganglion is at least 1,000 times greater than the amount released on nerve stimulation even in the presence of an anticholinesterase. Thus the dosage relationships of histamine may not be unlike those of acetylcholine.

The action of histamine in potentiating the effects of the ganglion blocking agents has its analogy in the results reported by Schenk and Anderson (1958) that histamine potentiates the action of certain of the neuromuscular blocking agents at the neuromuscular junction. These authors, however, observed a difference in action of histamine on the competitive as compared to the depolarizing neuromuscular blocking agents. Bovet-Nitti, Kohn, Marotta, and Silvestrini (unpublished observations), however, found that histamine potentiated both the competitive and depolarizing neuromuscular blocking agents *in vivo* in the rabbit and rat using the gastrocnemius and masseter muscle preparations. The experiments reported here for the ganglionic synapse appear to be more consistent with these latter findings, for we have found a similarity of action of histamine in enhancing both the competitive and the depolarizing ganglionic blocking agents. The physiological significance of these findings remains obscure.

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