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AN EFFECT OF HISTAMINE ON THE NICTITATING MEMBRANE OF THE CAT: POTENTIATION OF THE ACTIONS OF ADRENALINE, NORADRENALINE AND ACETYLCHOLINE*

BY

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It has been well established by experiments *in vivo* (Trendelenburg, 1954) and confirmed *in vitro* (Thompson, 1958) that histamine has no *direct* stimulating action on the nictitating membrane of the cat. The contractions of the nictitating membranes which do occur following an intravenous injection of histamine are produced indirectly as a result of at least two primary actions of this amine: the release of adrenal medullary catecholamines and stimulation of the superior cervical ganglia (Burn & Trendelenburg, 1954; Trendelenburg, 1954).

In addition to stimulating the ganglion cells, histamine facilitates ganglionic transmission of submaximal preganglionic stimuli (Trendelenburg, 1955) and also enhances the responses of the ganglion to injections of nicotine-like substances and potassium chloride (Konzett, 1952; Trendelenburg, 1956). On the other hand, the response of the nictitating membrane to submaximal post-ganglionic stimulation is unaffected by histamine (Trendelenburg, 1957). However, the influence of this amine on contractions produced by injected substances which stimulate the smooth muscle of the membrane has hitherto not been determined. In the present investigation the effect of histamine on contractions of the nictitating membrane produced by adrenaline, noradrenaline and acetylcholine has been investigated. Some of the results described herein have been reported to the American Society for Pharmacology and Experimental Therapeutics (Reit & Giarman, 1963).

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METHODS

The experiments were performed on cats of either sex weighing at least 2 kg. After inducing anaesthesia with pentobarbitone sodium (40 mg/kg intraperitoneally), the trachea was cannulated and most of the cats were rendered spinal as described by Burn (1952).

Contractions of the nictitating membrane were recorded semi-isometrically with the aid of a force-displacement transducer (Grass FT-03) coupled to an ink-writing polygraph (either Grass Model 5 or Gilson). The equilibrated resting load on the membrane was 3 *g*. In most experiments arterial blood pressure was recorded from the right femoral artery with a pressure transducer (Statham P23 AA) also coupled to the polygraph.

Close intra-arterial injections either to the nictitating membrane or to the superior cervical ganglion were made into the external carotid artery by means of a cannula tied into the central end of the lingual artery as described by Trendelenburg (1959). Drugs injected through this cannula are borne by the blood up the external carotid artery to the membrane unless the external carotid is clamped just distal to the origin of the lingual artery, in which case the injected drugs pass in a retrograde fashion down the external carotid into the arteries supplying the ganglion. The dead space in the cannula was 0.14 ml. Between drug injections the fluid retained in the dead space was usually aspirated and the cannula flushed twice with 0.2 ml. of 0.9% NaCl solution. However, when two injections—for example, of histamine and then acetylcholine—were to be made less than 1 min apart, the following procedure was routinely employed. First, 0.1 ml. of the histamine solution was introduced into the cannula. Since this volume was smaller than the dead space, the histamine remained within the cannula. Next the histamine was flushed out of the cannula into the external carotid artery by injecting 0.2 ml. of 0.9% NaCl solution. Finally, 0.24 ml. of the acetylcholine solution was injected, of which 0.1 ml., the excess over the dead space volume, entered the external carotid artery.

For intravenous injections a polyethylene cannula was tied into the left femoral vein. In most experiments heparin (0.5 mg/kg) was administered intravenously and both vagosympathetic trunks were cut low in the neck. For electrical stimulation of the cervical sympathetic nerve, it was separated from the vagus nerve, placed on bipolar platinum electrodes and covered with paraffin oil to prevent drying. Square wave stimuli of 0.5 msec duration were applied at various frequencies and intensities with a No. 104A Laboratory Stimulator (American Electronics Laboratories, Inc.).

In two cats, the right superior cervical and nodose ganglia were removed acutely; in two other cats, the ganglia were removed in an aseptic operation under pentobarbitone sodium anaesthesia, seven and 36 days before the actual experiments.

In three cats the medial smooth muscle of the left nictitating membrane was isolated, excised and mounted in 20 ml. organ bath containing Krebs solution in accordance with the procedure described by Thompson (1958). Drugs dissolved in 0.9% NaCl solution were injected into the bath in a volume of 0.1 ml. Between injections the bath was washed out twice and the muscle allowed to relax until the original baseline was re-attained. Contractions were recorded semi-isometrically with the aid of a force displacement transducer as for the nictitating membrane *in situ*; the equilibrated resting load on the muscle was 1 *g*.

Substances used: (−)-adrenaline-D-bitartrate, (−)-noradrenaline-D-bitrartrate, acetylcholine, chloride, histamine dihydrochloride, phentolamine methane sulphonate (Regitine-Ciba), *d*-tubocurarine (Intocostrin-Squibb), morphine sulphate, mepyramine maleate, bradykinin (BRS 640-Sandoz), adenosine, adenosine triphosphate, papaverine sulphate, isoxsuprine hydrochloride (Vasodilan–Mead Johnson), sodium nitrite, angiotensin (Hypertensin–Ciba) and vasopressin (Pitressin–Parke, Davies). All doses in the text refer to the base except that for morphine sulphate, expressed as the salt.

RESULTS

Histamine-induced potentiation

When injected into the external carotid artery adrenaline, noradrenaline and acetylcholine caused the nictitating membrane to contract. In contrast, histamine similarly injected either did not affect the membrane or made it relax slightly, but never caused it to contract. If, however, several seconds after the administration of the histamine one

of the three agonists was then injected, the contraction which occurred was greater than that produced by the same dose of the agonist injected before the histamine. Thus, the contractile effects of the agonists are potentiated by the histamine. This is illustrated in Fig. 1. In (b), (c) and (d) are shown records of the successive responses to pairs of intra-arterial injections of acetylcholine (10 μg), adrenaline (1 μg) and noradrenaline (3 μg). About 20 sec before the second injection of each of these agonists, 1 μg of histamine was injected by the same intra-arterial route and produced, as it did when injected in (a), a small relaxation of the membrane and a fall in blood pressure. Yet for each agonist it can be seen that the second contraction of the nictitating membrane elicited shortly after

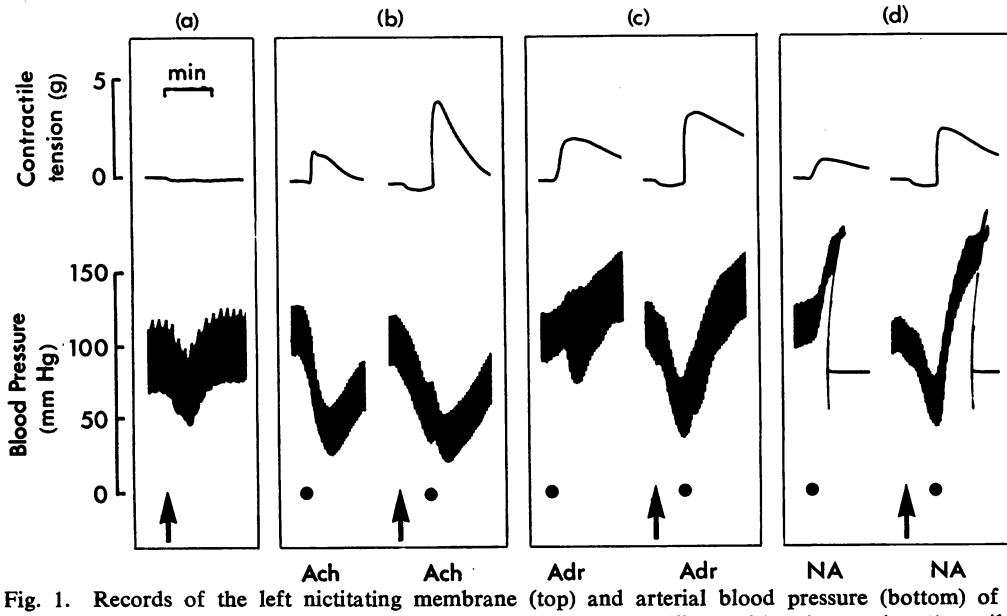

Fig. 1. Records of the left nictitating membrane (top) and arterial blood pressure (bottom) of a 3.5-kg cat anaesthetized with pentobarbitone sodium, immobilized with tubocurarine (1 mg/kg intravenously) and artificially ventilated. At the arrows intra-arterial injections toward the left nictitating membrane of 1 μg histamine. At the filled circles similar injections of 10 μg acetylcholine (Ach) in (b), 1 μg adrenaline (Adr) in (c) and 3 μg noradrenaline (NA) in (d). Record of blood pressure interrupted in (d) to avoid interference with record of nictitating membrane.

the histamine had been injected was greater than that produced before. The second response of the blood pressure, on the other hand, was in each case merely superimposed on that produced by the histamine and did not exhibit any net change in degree or direction. In several experiments the contractions of the nictitating membrane produced by adrenaline, noradrenaline and acetylcholine were enhanced by a prior injection of as little as 0.1 μg of histamine.

Ligation of the adrenal glands so that they were excluded from the circulation did not alter the histamine-induced enhancement of contractions evoked by adrenaline or acetylcholine. Moreover, an intravenous injection of phentolamine (2 mg/kg), which blocked the action of adrenaline and noradrenaline on the membrane, did not alter the histamine-induced potentiation of the contractile effect of acetylcholine. Thus, the

well-known ability of histamine to cause a release of catecholamines from the adrenal medulla (Burn & Dale, 1926) plays no part in its potentiating action at the nictitating membrane.

By injecting histamine (0.1–10 μg) toward the membrane, several attempts were made to influence contractions produced by submaximal stimulation of the ipsilateral cervical sympathetic nerve. These attempts were uniformly unsuccessful in agreement with the findings of Trendelenburg (1957a).

In the present investigation the production of potentiation by histamine or other substances was determined by comparing pairs of consecutive responses of the nictitating membrane to a given dose of one of the three agonists. As illustrated in Fig. 1, the first or control response of each pair was elicited before, and the second shortly after, an interposed injection of the potentiating substance. Potentiation is considered to have occurred if the second contraction was at least 25% larger than the first. However, even in those instances when the difference was less than 25%, the contractions elicited after an injection of histamine were usually somewhat larger than those elicited before. Only occasionally were they the same size, or rarer still, slightly smaller than the control contractions.

Potentiation was best demonstrated when the dose of the agonist employed produced control contractions which were no greater than one-half maximal. With such doses of the catecholamines, potentiation occurred in 69.3% (43/62) and 70.3% (26/37) of the trials for noradrenaline and adrenaline, respectively, while in the case of acetylcholine it occurred in 91.6% (392/428). In some experiments, histamine potentiated the contractile effects of acetylcholine but not those of adrenaline or noradrenaline. The converse, however, did not hold true—that is, when potentiation failed to occur for acetylcholine, it also failed to occur for the catecholamines. As an agonist acetylcholine had another advantage over the catecholamines in that the nictating membrane recovered more rapidly from its effects, permitting injections to be made more frequently. Hence in most of the experiments acetylcholine was the agonist employed.

Effect of the intervals between injections of histamine and acetylcholine

Potentiation occurred only when the injection of histamine and of agonist were made separately. This is illustrated by the experiment shown in Fig. 2. In (b) when acetyl-

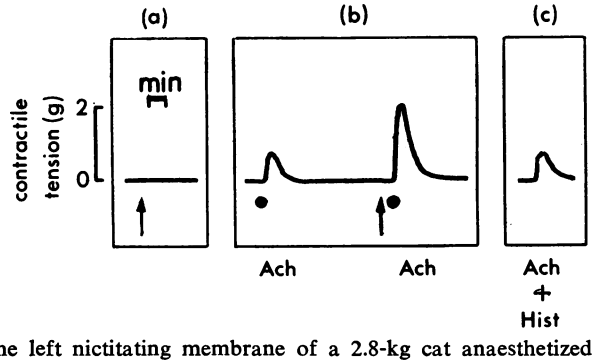

Fig. 2. Record of the left nictitating membrane of a 2.8-kg cat anaesthetized with pentobarbitone sodium and artificially ventilated. At the arrows intra-arterial injections toward the left nictitating membrane of 1 μg histamine. At the filled circles similar injections of 10 μg acetylcholine (Ach) in (b) and a mixture of 10 μg acetylcholine plus 1 μg histamine (Ach+Hist.) in (c).

choline (10 μg) was injected about 20 sec after histamine (1 μg) it caused a contraction which was more than twice as large as the one it elicited before histamine. However, when the same quantities of these two substances were combined in a single injection (c) the response of the nictitating membrane was the same as that produced by 10 μg of acetylcholine alone.

The degree of enhancement produced depended upon the length of time between the injection of histamine and the subsequent injection of acetylcholine. The results of two experiments in which this injection interval was varied randomly are plotted in Fig. 3. In the one represented by the solid line there was a small increase in tension with an injection interval as short as 4 sec, but maximal enhancement required an interval of 15 sec. With longer intervals the degree of enhancement gradually declined until with an interval of 60 sec, potentiation no longer occurred. In the experiment represented by the broken line, the same type of curve was obtained although shifted somewhat to the right, especially at the point of maximal enhancement, which occurred in this case at 26 sec. These results show that the potentiating effect of histamine takes several seconds to develop, reaches its maximum fairly rapidly, is dissipated more slowly and finally disappears in about a minute. In all studies of the potentiation effect other than those summarized in Fig. 3 an interval of about 20 sec between the injection of histamine and of agonist was routinely employed.

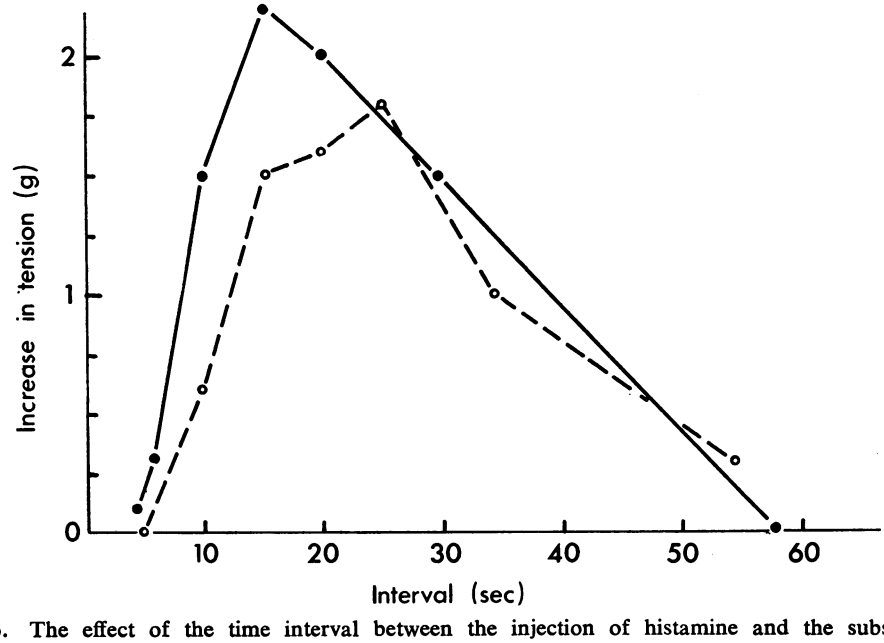

Fig. 3. The effect of the time interval between the injection of histamine and the subsequent injection of acetylcholine on the degree of enhancement of the response of the nictitating membrane to acetylcholine. The intervals (sec) were measured from the end of the injection of histamine (1 μg) to the end of the injection of acetylcholine. The degree of enhancement is expressed as the net increase in tension (*g*) of each potentiated response over its control. (●——●) 3-kg spinal cat; left superior cervical and nodose ganglia removed at the beginning of the experiment. Contractions of the left nictitating membrane were produced with 3 μg doses acetylcholine. (○——○) 3.8-kg spinal cat; ganglia intact. Contractions of the left nictitating membrane were produced with 10 μg doses acetylcholine.

Effect of histamine on the time course of responses

In addition to altering the size of the responses of the nictitating membrane to adrenaline, noradrenaline and acetylcholine, histamine also altered the time course of these responses. Thus, in contrast to the control contractions, those elicited after the histamine began sooner after injection of the agonist, and attained their peak and usually subsided at a faster rate. As an index for comparison of these temporal differences between control and potentiated responses, in some experiments we measured the time that elapsed between the end of the injection of the agonist and the beginning of the recorded contraction. For control responses, this latent period varied from 1.5 to 22 sec. For the responses potentiated by histamine, it was decreased in most of the trials (131/150) with acetylcholine as the agonist, and in all of the trails with the catecholamines as agonists (6/6 with noradrenaline and 12/12 with adrenaline). The reduction in latency varied from 0.5 to as much as 14 sec, but there was no consistent relationship between the degree of reduction in latency and the degree of enhancement of the responses by histamine.

Analysis of the site of action of histamine

Substances injected into the external carotid artery of spinal cats which affect the responsiveness of the nictitating membrane may act at several possible sites including: (1) neighbouring structures in the orbit, (2) the smooth muscle of the membrane itself, (3) its nerve supply, and/or (4) its blood supply. By a systematic analysis, all but the last possibility, an action on the blood supply of the nictitating membrane, have been excluded. The evidence is as follows:

Neighbouring structures. After ablation of the eyeball and dissection of the nictitating membrane free from most of its fascial attachments, potentiation by histamine of the contractions elicited by acetylcholine and the catecholamines was readily produced. Hence the potentiation could not be due to an action of histamine on the extrinsic ocular muscles, skeletal muscles to some of which the nictitating membrane is known to be anatomically connected (Acheson, 1938).

The smooth muscle. In experiments on the isolated smooth muscle of the nictitating membrane *in vitro*, histamine was without effect. In concentrations of 0.1–5 μg/ml. it neither altered the resting state of the smooth muscle nor enhanced the contractile effects of acetylcholine (0.1–1 μg/ml.). These results are in agreement with the findings of Thompson (1958).

Nerve supply. Histamine, by a direct action on the ganglion cells, is known to stimulate the sympathetic fibres which innervate the nictitating membrane (Trendelenburg, 1954). However, this action of histamine in no way contributes to its potentiating effect on responses of the smooth muscle of the nictitating membrane to acetylcholine. In several cats in which the ipsilateral superior cervical and nodose ganglia were removed, histamine still enhanced the response of the nictitating membrane to acetylcholine. This occurred whether the ganglia were removed acutely (see Fig. 3) or more than six days before the actual experiment.

Morphine, which is known to block the stimulating action of histamine on sympathetic ganglia (Trendelenburg, 1957b), did not affect the histamine-induced potentiation at the

nictitating membrane (Fig. 4). In (a) the response to 3 μg of acetylcholine was enhanced in typical fashion by 1 μg of histamine injected toward the membrane. A similar injection of histamine alone (b) produced only a slight relaxation of the membrane, but the same dose injected in a retrograde fashion toward the superior cervical ganglion while the external carotid artery was clamped (c), stimulated the ganglion cells, thereby causing a small contraction. After an intravenous injection of 1 mg morphine sulphate the ganglion-stimulating action of histamine was abolished (compare (e) with (c)), whereas the potentiating action of histamine at the membrane was unaffected (compare (d) with (a)).

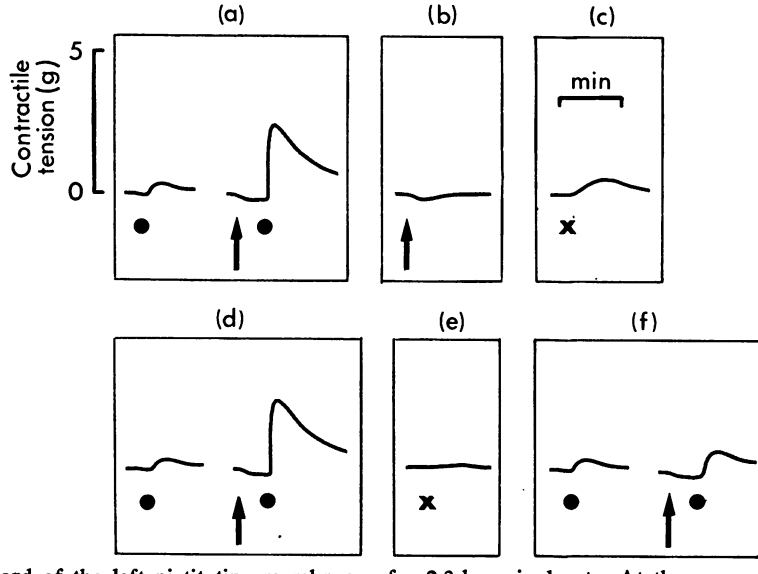

Fig. 4. Record of the left nictitating membrane of a 2.3-kg spinal cat. At the arrows intra-arterial injections towards the left nictitating membrane of 1 μg histamine. At the filled circles, similar injections of 3 μg acetylcholine. At the crosses, retrograde intra-arterial injections towards the left superior cervical ganglion of 1 μg histamine. Between (c) and (d) intravenous injection of 1 mg morphine sulphate. Between (e) and (f) intra-arterial injection towards the left nictitating membrane of 10 μg mepyramine.

In contrast to morphine, mepyramine, which antagonizes the action of histamine on most smooth muscle preparations as well as on ganglia, inhibited the histamine-induced potentiation at the nictitating membrane. In the same experiment (Fig. 4) after an intra-arterial injection of 10 μg mepyramine toward the membrane the degree of enhancement by histamine was greatly diminished (compare (f) with (a) and (d)).

These results exclude any possible involvement of the ganglion-stimulating action of histamine in its ability to potentiate the effects of acetylcholine on the nictitating membrane. In addition, they indicate that to produce potentiation, histamine acts upon specific receptors which are similar to those upon which it acts in histamine-sensitive smooth muscle, but not in ganglia.

Blood supply. Histamine is a potent vasodilator. If this action were responsible for its ability to potentiate the effects of injected agonists on the nictitating membrane, other

vasodilator substances should also cause potentiation. Vasoconstrictor substances, on the other hand, would be expected not to potentiate, but rather to exert an inhibitory influence. These predictions were borne out by the results of experiments in which other vasoactive substances were injected instead of histamine.

Bradykinin, a polypeptide with actions on the vascular system similar to those of histamine, also potentiated the contractile effects of acetylcholine. In the experiments shown in Fig. 5, the enhancement produced by 10 μg bradykinin (c) was somewhat greater than that produced by 1 μg histamine (a). When bradykinin alone was injected toward the membrane (b), like histamine is caused only a slight relaxation.

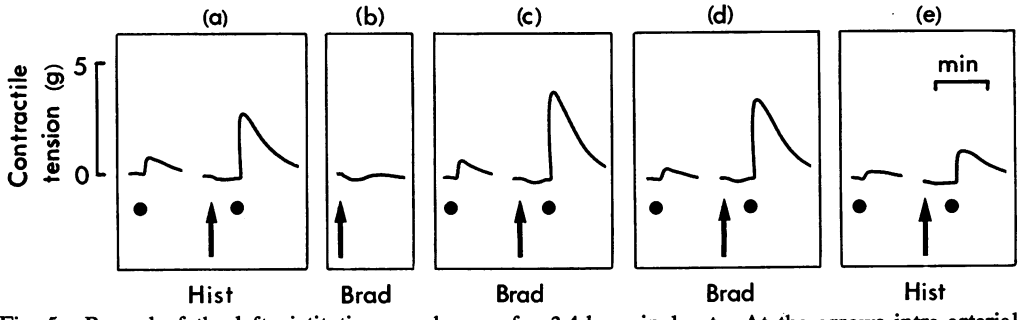

Fig. 5. Record of the left nictitating membrane of a 3.4-kg spinal cat. At the arrows intra-arterial injections towards the left nictitating membrane of 1 μg histamine (Hist.) in (a) and (e), and 10 μg bradykinin (Brad) in (b), (c) and (d). At the dots, similar injections of 3 μg acetylcholine. Between (c) and (d) a similar injection of 10 μg mepyramine.

Although the potentiating effects of bradykinin and histamine appear to be similar, the receptors with which these two substances interact are different. In the experiment shown in Fig. 5, after the administration of 10 μg mepyramine toward the membrane, the potentiation by bradykinin was barely affected, whereas that by histamine was considerably decreased (compare (d) with (c) and (e) with (a)).

In addition to bradykinin, several other vasodilator substances were studied which also potentiated the effect of acetylcholine on the nictitating membrane. When injected alone, none of them caused the membrane to contract, although in some instances they caused it to relax slightly. Calculated from the threshold doses which produced potentiation, the molar potencies of these substances in relation to histamine are as follows (histamine-100): bradykinin, 97; adenosine, 24.5; adenosine triphosphate, 13.5; papaverine, 0.3; isoxsuprine, 0.15; sodium nitrite, 0.01. Hence, on a molar basis only bradykinin approaches histamine in effectiveness as a potentiator.

Rather than potentiating the response of the nictitating membrane to acetylcholine, the vasoconstrictors, angiotensin and vasopressin, had just the opposite effect. In the experiment shown in Fig. 6, the inhibition produced by 0.1 μg angiotensin (b) and 0.2 u. (0.5 μg) of vasopressin (c) contrasted sharply with the potentiation produced by 1 μg histamine (a).

In addition to the administration of vasodilator drugs intra-arterially, another method of producing relaxation of the blood vessels in a given anatomical region is by making the region ischaemic. In the cat, for example, the vascular beds of limbs and intestine are known to undergo marked dilatation within seconds after their major arteries have

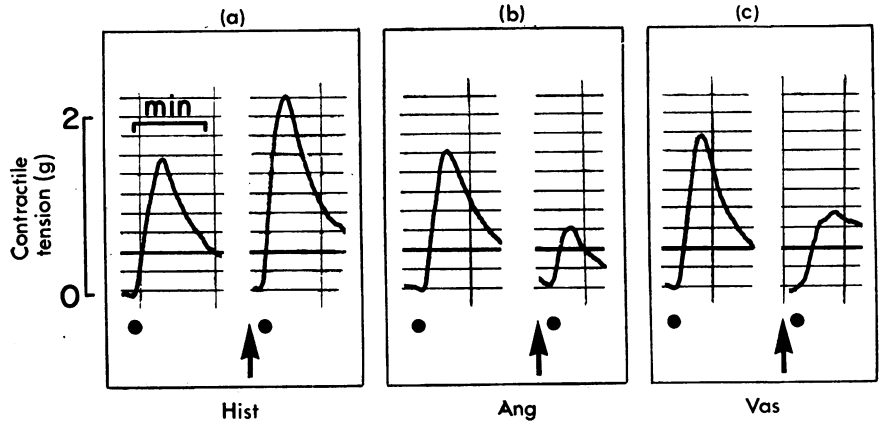

Fig. 6. Record of the right nictitating membrane of a 2.3-kg spinal cat. At the arrows intra-arterial injections toward the right nictitating membrane of 1 μg histamine (Hist) in (a), 0.1 μg angiotensin (Ang) in (b) and 0.2 u. vasopressin (Vas) in (c). At the filled circles similar injections of 0.1 μg acetylcholine.

been clamped. After restoration of arterial blood flow a transient reactive hyperaemia occurs which is indicative of the profound decrease in regional peripheral resistance that had been produced. Following the hyperaemia, the normal degree of vasomotor tone rapidly becomes re-established (Bayliss, 1902; Folkow, 1949; Thulesius, 1962). We therefore studied the effect of temporary occlusion of the external carotid artery on responses of the ipsilateral nictitating membrane to acetylcholine and to sympathetic stimulation. The manipulations of clamping and unclamping the artery did not appear to exert any pronounced effects on the muscle tension of the membrane. When the clamp was applied, a small contraction occurred; when the clamp was released, a small relaxation occurred (Fig. 7b). However, after a short period of arterial occlusion, the contractile effect of acetylcholine was enhanced; a given dose injected toward the membrane just as

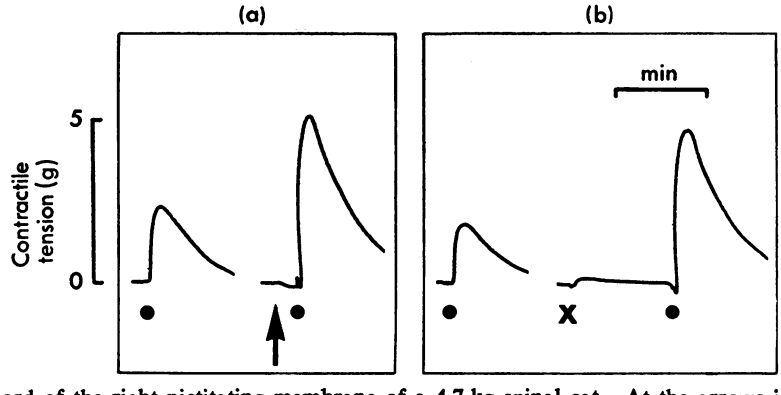

Fig. 7. Record of the right nictitating membrane of a 4.7-kg spinal cat. At the arrows intra-arterial injection to the right nictitating membrane of 1 μg histamine. At the filled circles, similar injections of 10 μg acetylcholine. At the cross, occlusion of the external carotid artery just distal to the origin of the lingual artery with a small bulldog clamp. The clamp was removed just as the second injection of acetylcholine was made in (b).

the arterial clamp was released elicited a much larger contraction than that dose did before the clamp was applied. In the experiment of Fig. 7, the potentiation brought about by occlusion of the external carotid artery for 60 sec (b) was comparable to that produced by 1 μg histamine (a). Mepyramine (1 mg/kg), injected intravenously, abolished the histamine-induced potentiation, but had no effect on that caused by arterial occlusion.

Temporary occlusion of the external carotid artery for periods of 5 to 120 sec failed to enhance responses of the nictitating membrane to nerve stimulation. The contractions elicited immediately after unclamping the artery either were no different or somewhat smaller than those elicited before the clamp was applied.

DISCUSSION

The results of this investigation show that contractions of the nictitating membrane *in situ* evoked by acetylcholine, adrenaline and noradrenaline may be enhanced by a prior intra-arterial injection of histamine directed toward the membrane. In contrast, as reported by Trendelenburg (1957a) and confirmed in our laboratory, similar injections of histamine have no effect on the contractions evoked by stimulating the cervical sympathetic nerve. Furthermore, contractions of the isolated nictitating membrane *in vitro* produced by acetylcholine are also unaffected by histamine. Thus, in order for histamine to exert its potentiating action, the contractions of the nictitating membrane have to be produced by substances that reach the smooth muscle of the membrane *via* its blood supply.

Among the many biological properties of histamine there are several which might have accounted for its potentiating effect. For example, histamine excites sympathetic ganglion cells, and even subthreshold amounts of this amine facilitate transmission in the superior cervical ganglion of the cat (Burn & Trendelenburg, 1954; Trendelenburg, 1954). In the present investigation, we attempted to avoid interference from the ganglion-stimulating action of histamine by injecting it *via* the lingual artery into the external carotid and therefore distal to the arteries supplying the ganglion which arise near the bifurcation of the carotid (Davis & Story, 1943; Chungcharoen, de Burgh Daly & Schweitzer, 1952). For optimal potentiation, however, it was necessary to inject the histamine about 20 sec before administration of the agonists—ample time, according to the data of Gray & Paton (1949), for recirculation of injected substances to occur in the cat. Conceivably, on recirculation, some of the histamine may have reached the ganglion and caused a small release of sympathetic neurotransmitter from nerve terminals in the nictitating membrane, a release insufficient *per se* to produce a contraction but still adequate to sum with the subsequently injected submaximal doses of catecholamines or acetylcholine so that their contractile effects appeared to be enhanced. This possibility, however, is most unlikely since potentiation at the membrane was unaffected by removing the ganglion or by injecting morphine in a dose which blocked the ganglion-stimulating action of histamine. The failure of morphine to inhibit the potentiation also makes it unlikely that histamine was acting on ganglion cells in the post-ganglionic sympathetic nerve trunk that are distal to the superior cervical ganglion (Boyd, 1957), since presumably morphine would block the stimulating action of histamine on such cells as well as on those situated within the ganglion.

Another property of histamine which might have accounted in part for its potentiating effect is its ability to cause a release of catecholamines from the adrenal medulla (Burn & Dale, 1926). Even subthreshold amounts of medullary catecholamines in the blood might well augment the contractile effects of submaximal doses of adrenaline, noradrenaline or acetylcholine on the nictitating membrane. This mechanism was in fact cited by Schmiterlöw (1951) to account for his observations that, in cats pretreated with cocaine, intravenous injections of mixtures of histamine and noradrenaline or adrenaline produced larger contractions of the nictitating membrane than injections of either catecholamine alone. In our experiments, however, the potentiating effect of histamine cannot be explained in terms of a release of medullary catecholamines, because after the suprarenal glands were excluded from the circulation, potentiation of the contractile effects of acetylcholine and adrenaline were undiminished. Still, there remained the possibility that low levels of circulating catecholamines might have been liberated by histamine from extramedullary stores. This possibility, however, was rigorously excluded by the use of the alpha-receptor blocking drug phentolamine, which did not affect the histamine-induced potentiation of the contractile effects of acetylcholine, although it abolished the effects of adrenaline and noradrenaline at the membrane.

In order to reach their receptors in the nictitating membrane from the blood stream the acetylcholine, adrenaline and noradrenaline must first enter the capillaries in which exchanges between the blood and the tissue fluid take place. A characteristic feature of the capillaries in various organs of mammals and amphibia is that under resting conditions large numbers of them are closed, and thus there is normally a considerable reserve of potentially functional exchange surface (Krogh, 1929). In the absence of any evidence to the contrary there is no reason to assume that this does not also apply to the capillary bed in the nictitating membrane of the cat. Obviously, the proportion of blood-borne molecules of agonist that reach receptors in the membrane can be influenced by the number of open capillaries they encounter. Hence, if there was an increase in total capillary patency, a given dose of agonist might reasonably be expected to evoke a larger contraction than it would under normal resting conditions. Furthermore, with more capillaries open it would take less time for a threshold level of an agonist to accumulate at the receptors and therefore the contraction in addition to being larger should begin sooner. This describes the contractions produced by acetylcholine, adrenaline and noradrenaline after an injection of histamine, which, in addition to its other properties, is also a potent capillary vasodilator (Dale & Richards, 1918).

The finding that histamine potentiated the contractile effects of acetylcholine more consistently than those of the catecholamines is compatible with the view that the potentiating action of histamine at the nictitating membrane is a function of its local vasodilating effect since acetylcholine would tend to supplement the vasodilator action of histamine, whereas the catecholamines through their vasoconstrictor action would tend to counteract it. Also consistent with a potentiation mechanism involving vasodilatation within the nictitating membrane are the findings that under experimental conditions identical to those employed for histamine, other vasodilators also produced potentiation, whereas vasoconstrictors produced inhibition. Among the vasodilators tested, bradykinin, the one most closely comparable in vasodilating potency to histamine in other experimental situations (Elliott, Horton & Lewis, 1960), was the only one which approached histamine in efficacy. In this connexion it also may be pertinent that of the

vasodilators studied only bradykinin further resembles histamine in its ability to increase capillary permeability. Although bradykinin produced potentiation and angiotensin produced inhibition, it is noteworthy that both of these peptides are known to stimulate the superior cervical ganglion (Lewis & Reit, 1965) and to release catecholamines from the adrenal medulla (Feldberg & Lewis, 1964). This constitutes additional evidence against the possibility that an action at either of these sites might be involved in the potentiation observed at the nictitating membrane.

In addition to drug-induced vasodilatation, temporary occlusion of the external carotid artery potentiated the contractile effects of acetylcholine on the nictitating membrane. This procedure, by analogy with the effect of transient ischaemia on the vascular beds of other organs (see especially Thulesius, 1962), must have caused a prompt and profound relaxation of the vascular smooth muscle in the nictiating membrane. The acetylcholine injected immediately after the external carotid artery was unclamped would have entered the arterial blood supplying the membrane at a moment when the reactive hyperaemia was at its peak and thus would have been distributed rapidly throughout the widely dilated capillary bed. The enhanced contraction of the nictiating membrane observed under these conditions provides support for the view that the potentiating effect of histamine may be contingent upon its ability to increase blood flow into the capillaries of the membrane. This view, however, must be regarded as tentative until it can be validated by direct observation of the microcirculatory changes that occur in the nictitating membrane in conjunction with the enhanced contractile responses of this organ.

SUMMARY

1. The effect of histamine on contractions of the nictitating membrane of the cat produced by adrenaline, noradrenaline and acetylcholine was investigated. For this purpose, close intra-arterial injections of these substances were made to the membrane through the central end of the cannulated lingual artery.

2. Injected in this way, each of the three agonists produced larger contractions just after an injection of histamine than before it. Hence, their contractile effects were potentiated by the histamine. This occurred most consistently when acetylcholine was the agonist employed.

3. The threshold dose of histamine for producing potentiation was 0.1 μg.

4. The degree of potentiation varied according to the time interval between the injection of histamine and the subsequent injection of acetylcholine. It was maximal when they were given about 20 sec apart. With intervals shorter than 4 sec or longer than 60 sec, there was no potentiation.

5. For each agonist, histamine affected not only the size but also the time course of the responses, which began after a shorter latency and attained their peak and usually subsided at a faster rate than the control responses.

6. Two vasoconstrictor substances (angiotensin and vasopressin) injected into the membrane in place of histamine inhibited the contractile effects of acetylcholine. In contrast, several vasodilators (bradykinin, adenosine, adenosine triphosphate, papaverine, isoxsuprine and sodium nitrite) produced potentiation. In this respect only bradykinin approached histamine in potency on a molar basis.

7. Temporary occlusion of the external carotid artery potentiated the contractile effects of acetylcholine injected immediately after the artery was unclamped.

8. Mepyramine specifically antagonized the potentiating effect of histamine.

9. The potentiating effect of histamine is independent of its ability to stimulate the superior cervical ganglion or to release catecholamines from the adrenal medulla or other storage sites, but is believed to be due to its ability to produce capillary vasodilatation within the nictitating membrane.

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