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THE EFFECT OF MORPHINE ON THE ADRENERGIC NERVES OF THE ISOLATED GUINEA-PIG JEJUNUM

BY

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Nerves accompanying the mesenteric blood-vessels of the isolated guinea-pig jejunum were stimulated and changes in the longitudinal muscle coat were recorded. Stimulation of the nerves led to a rapid relaxation if the intestine was contracted by a previous administration of histamine. The relaxation was inhibited by morphine and partially restored by nalorphine. Bretylium and atropine also inhibited the relaxation and cocaine increased it somewhat. Morphine, atropine, and bretylium had no significant effect on the depressant action of noradrenaline on histamine contractions, whereas cocaine slightly enhanced the action of noradrenaline. Morphine, but not the other drugs, prevented the inhibitory action of dopamine.

Morphine and other narcotics inhibit contractions due to postganglionic cholinergic stimulation, by reducing the release of acetylcholine from nerve endings in the isolated guinea-pig intestine (Paton, 1957). Morphine decreases the cardiac slowing produced by vagal stimulation in the rabbit, rat, and to some extent in the cat (Kosterlitz & Taylor, 1959). Interference by morphine with the release of noradrenaline from the adrenergic nerve endings of the cat nictitating membrane has also been reported by Trendelenburg (1957). This paper describes the action of morphine on the adrenergic nerves of the isolated guinea-pig jejunum. In order to obtain a fuller understanding of the mechanism of action of morphine on this relatively simple preparation, other agents known to affect autonomic nerves were also included in this study.

METHODS

For stimulating the nerves leading to the intestine a preparation similar to that described by Finkleman (1930) for the rabbit gut was used. Freshly fed male guinea-pigs, weighing between 350 and 500 g, were killed by a blow on the head and 3 to 5 cm long pieces from the oral part of the jejunum were excised along with the attached mesentery. After washing the lumen of the intestine, the mesentery containing the artery supplying the gut was attached to shielded, silver, bipolar electrodes. The preparation including the electrodes was immersed in a 50 ml. bath, in such a way as not to interfere with the contraction or relaxation of the longitudinal muscle coat. Nerves accompanying the blood-vessels in the mesentery were stimulated by means of an Arthur H. Thomas square-wave stimulator, with impulses of 0.5 msec duration and with frequencies described in the section on results. There was no need for any further insulation of the electrodes from the bath fluid, as maximal relaxation from stimulation could be obtained by setting the output at 5 to 20 V. If the electrodes were immersed in the bath without the mesentery attached to them, or when the mesentery was tied between the electrodes and the intestine, electrical impulses that produced maximal relaxation before became ineffective. This showed that the impulses reached the intestine only through the mesenteric nerves.

Stimulation of the periarterial nerves of a quiescent piece of intestine led to a biphasic response, consisting of an initial brief contraction followed by a more prolonged relaxation, similar to that described by Munro (1953). In order to investigate how drugs affect the relaxation of the longitudinal muscle coat brought about by stimulating the mesenteric nerves, it was found necessary to apply the stimulus when the muscle was contracted by the addition to the bath of acetylcholine or histamine. Otherwise, the inhibition produced by depressant drugs would have precluded any further relaxation. In preference to acetylcholine, histamine was chosen to increase the tonus of the intestine, because the inhibition resulting from stimulation was greater in the case of histamine. Moreover, the use of histamine allowed the action of atropine to be observed. In order to maintain the tonus of the intestine at about constant level at the time of stimulation, histamine contractions had to be alternated with periods of rest. Without these rest periods the tonus of the intestine declined rapidly, in spite of the continued presence of histamine. For this reason the following procedure was adopted: every 4 min the intestine was exposed to 0.8 to 4×10^{-7} histamine which produced nearly maximal contraction. One min later, when the intestine had reached a constant level of contraction (that could be taken as a base-line for assessing the effect of nervous stimulation) the mesenteric nerves were stimulated for 5 or 15 sec. Following the relaxation resulting from stimulation, the intestine usually regained its pre-stimulation tonus by the time histamine was washed out of the bath, 2 min after its administration. The degree of relaxation produced by stimulation remained constant when this cycle of events was repeated many times. It was noted, however, that pieces from the more anal parts of the small intestine did not maintain a constant level of contraction for even 2 min, whereas the more oral parts (jejunum) responded to histamine with a steady contraction for this length of time. In addition, the jejunum proved to be more sensitive to the effects of morphine, and for these reasons it was used in all experiments described in this paper.

In the second part of the experiments the depressant effect of sympathomimetic amines on the contractions produced by histamine was measured. The intestine, suspended in a 10 ml. bath, was exposed to a constant amount of histamine (0.2 to 0.5 μg) every 3 min for 30 sec. The bath was then flushed for 20 sec. These operations were performed automatically by means of two Fisher electro-hosecocks connected to relays. The relays were activated by contacts made through perforations in the paper of a kymograph drum, revolving every 3 min.

Contractions of the longitudinal muscle coat were recorded by means of a light, isotonic, frontal lever, giving a magnification of 4:1. A Tyrode solution containing 8 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 0.09 g $MgSO_4$, 1 g $NaHCO_3$, and 1 g glucose per l. and aerated with a mixture of 95% oxygen and 5% carbon dioxide was employed throughout. All experiments, except those specifically mentioned, were performed at 37° C bath temperature. Morphine sulphate, bretylium tosylate, cocaine hydrochloride, atropine sulphate, hexamethonium chloride or pentolinium tartrate was dissolved in the Tyrode solution contained in the reservoir, while nalorphine hydrochloride, acetylcholine bromide, noradrenaline, dopamine (3-hydroxytyramine) hydrochloride, and ephedrine sulphate were added to the bath by means of syringes. Nalorphine was dissolved in Tyrode, acetylcholine in 0.9% NaCl, whereas noradrenaline, dopamine, or ephedrine was dissolved in distilled water containing 1 mg % ascorbic acid and injected in a constant volume of 0.1 ml. Histamine acid phosphate, dissolved in Tyrode solution, was administered with a syringe in experiments involving nervous stimulation, whereas in the experiment described in the second part, histamine solution was delivered into the bath from a small Marriotte bottle. All drug concentrations, with the exception of those of noradrenaline, hexamethonium, and pentolinium, refer to the salt form and are expressed in units of g/ml.

RESULTS

Stimulation of adrenergic nerves

Morphine. The relaxation resulting from mesenteric nerve stimulation of the jejunum, with a voltage about 50% above maximal, was reduced or abolished by 10^{-8} to 10^{-6} morphine. As shown in Fig. 1, replacing the bath fluid with one

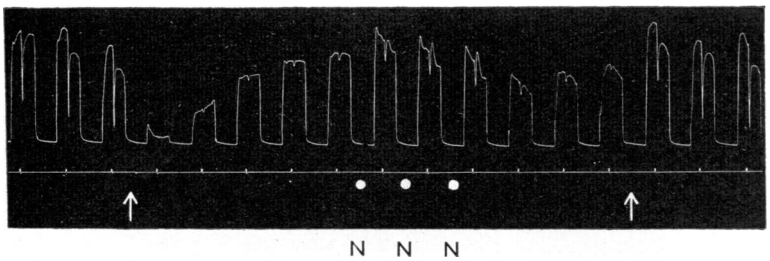

Fig. 1. Effect of morphine and nalorphine on the inhibition produced by mesenteric nerve stimulation. Every 4 min 20 μg histamine added, followed 1 min later by supramaximal stimulation with 20 pulses/sec for 5 sec. Between arrows 10^{-7} morphine in the bath fluid; at N 0.5 μg nalorphine added to the bath. Bath vol. 50 ml.

containing morphine, first considerably reduced the height of histamine contractions. At the same time, the effect of stimulation also decreased. Later, the histamine contractions returned to normal, whereas the relaxation due to stimulation was still diminished. Following the removal of morphine, the effectiveness of stimulation gradually returned. No evidence was found that morphine was more effective when submaximal voltage was employed or the frequency of stimulation was decreased.

Nalorphine. Nalorphine alone reduced the effect of adrenergic stimulation only slightly in a concentration of 10^{-6}, whereas smaller concentrations were without an effect. However, in the presence of morphine (10^{-7}) concentrations of nalorphine ranging from 2×10^{-9} to 2×10^{-7} increased the effect of adrenergic stimulation (Fig. 1). The antagonism of nalorphine to morphine was rather weak: it could be shown only when morphine did not fully inhibit the effect of stimulation. Even when nalorphine antagonized the action of morphine, it failed to restore fully the effectiveness of stimulation. Frequently, nalorphine increased the size of histamine contractions which were depressed by morphine. It was not possible to obtain quantitative data on the optimal concentration of nalorphine required to antagonize any given concentration of morphine because the action of morphine varied greatly from one preparation to the other. In various experiments, nalorphine was found to be an effective antagonist when present in a concentration of 1/50 to twice that of morphine.

Bretylium. This drug has been shown to reduce selectively the effect of adrenergic stimulation by preventing the release of noradrenaline from nerve endings (Boura & Green, 1959). It has been included in this study to ascertain the nature of the inhibition resulting from mesenteric nervous stimulation. Bretylium 10^{-6} progressively reduced the relaxation due to stimulation, until in about 30 min the inhibition became complete (Fig. 2). Washing out of the drug from the bath restored the relaxation only very slowly.

Atropine. The effect of atropine was tested because it was thought that by eliminating the cholinergic effect of stimulating a mixed nerve, it would increase the inhibition produced by the stimulation. Surprisingly, in even small concentrations (10^{-8}), atropine prevented the relaxation due to stimulation (Fig. 3). The

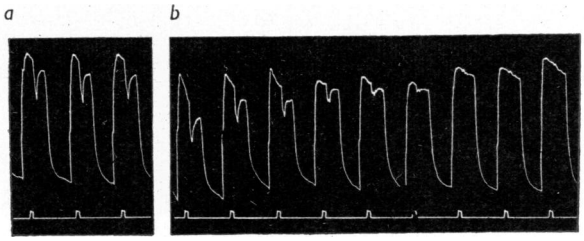

Fig. 2. Effect of bretylium on the inhibition due to mesenteric nerve stimulation. Every 4 min 20 μg histamine added, 1 min later supramaximal stimulation with 20 pulses/sec for 15 sec. At the beginning of (*b*) bretylium 10^{-6} added to the bath fluid. Bath vol. 50 ml.

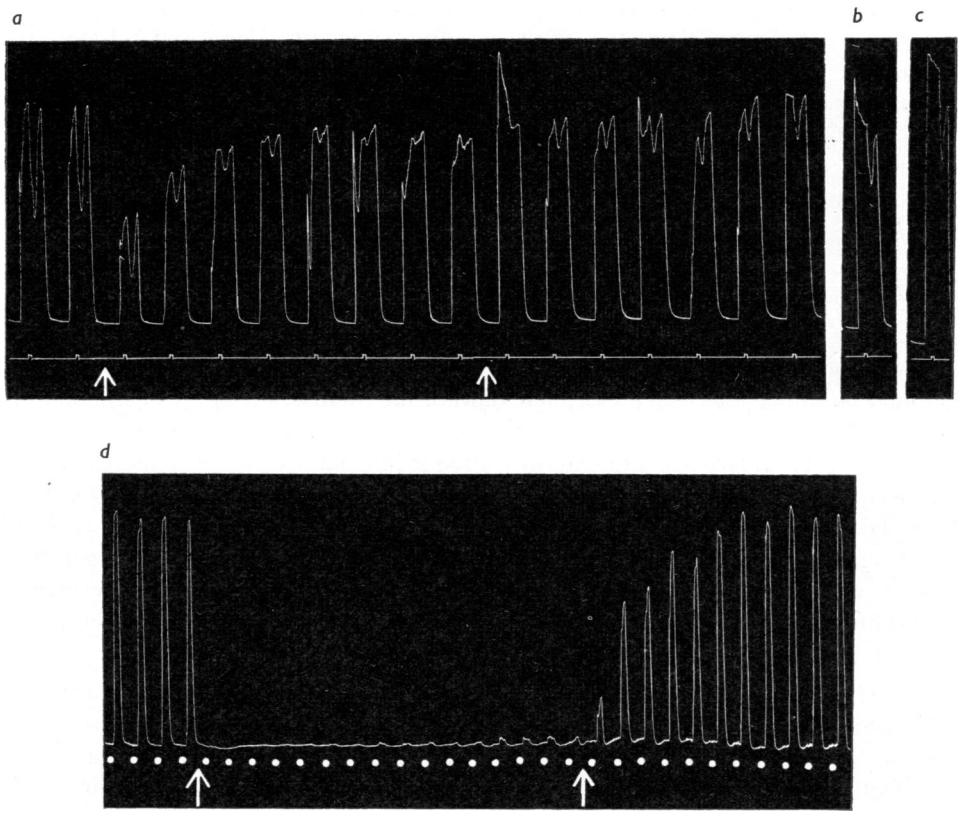

Fig. 3. (*a*) Effect of atropine on the inhibition due to mesenteric nerve stimulation. Every 4 min 10 μg histamine added, 1 min later supramaximal stimulation with 20 pulses/sec for 15 sec. Between arrows 10^{-8} atropine present in the bath fluid. (*b*) 32 min after *a*; (*c*) 16 min after *b*. (*d*) Effect of atropine on acetylcholine contractions. Every 2 min 2 μg acetylcholine added to the bath. Between arrows 10^{-8} atropine present in bath fluid. Bath vol. 50 ml.

inhibition was slow in onset and, once established, was slow to disappear after the drug has been removed from the bath. This course of events was entirely different from the antagonism of acetylcholine by atropine (Fig. 3*d*). Contractions caused by acetylcholine were immediately inhibited and returned to normal soon after atropine was washed out.

Cocaine and hexamethonium. Cocaine, in a concentration of 0.5 to 1×10^{-5}, increased the relaxation due to submaximal stimulation and prolonged somewhat the relaxation from supramaximal stimulation (Fig. 4). On the other hand, hexamethonium (1 to 2×10^{-5}) was without effect.

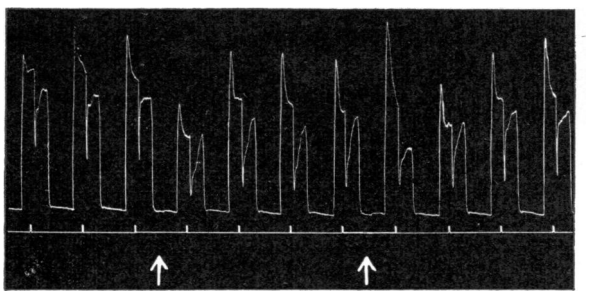

Fig. 4. Effect of cocaine on the inhibition produced by mesenteric nerve stimulation. Every 4 min 4 μg histamine added, followed 1 min later by supramaximal stimulation with 20 pulses/sec for 5 sec. Between arrows 0.5×10^{-5} cocaine present in bath fluid. Bath vol. 50 ml.

Action of sympathomimetic amines

Certain sympathomimetic amines act indirectly, through the release of noradrenaline from adrenergic nerve endings (Burn & Rand, 1958a). This observation afforded another method for assessing the action of morphine on the release of noradrenaline. In addition to noradrenaline, tyramine, dopamine, ephedrine and amphetamine were tested for their ability to inhibit histamine contractions. Of these tyramine and amphetamine stimulated the intestine and therefore had to be discarded. The other three amines had a purely depressant effect.

In order to measure the inhibitory action of the sympathomimetics it was necessary to obtain constant contractions to repeated submaximal doses of histamine. At 37° C, in the absence of any drug, the intestine showed considerable spontaneous activity and the responses to histamine varied a great deal. By adding 0.5×10^{-5} pentolinium to the bath fluid, however, the responses to histamine became more regular. Under these conditions exposing the intestine for 2 min to 2×10^{-8} noradrenaline or to 2×10^{-6} dopamine reduced the height of the subsequent histamine contraction by 10 to 70%, dopamine being somewhat more depressant than noradrenaline on the same piece of jejunum. Following the washing out of the sympathomimetic amine and the subsequent addition of histamine, the intestine often contracted, as shown in Fig. 5. As the experiment progressed, the intestine became more sensitive to the inhibitory effect of the sympathomimetics.

Introduction of 10^{-7} morphine into the bath fluid first greatly depressed the size of histamine contractions. After the response to histamine had become stabilized,

somewhat below the control level, dopamine inhibited only slightly the histamine contractions, whereas the depression produced by noradrenaline was affected much less by the presence of morphine (Fig. 5). Not only was the relative reduction

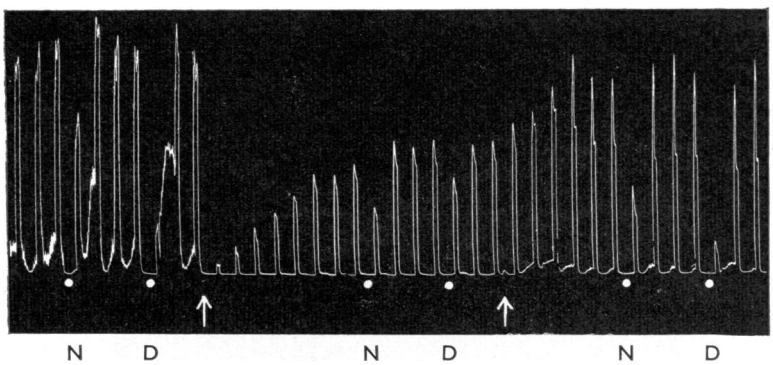

Fig. 5. Effect of morphine on the inhibitory action of noradrenaline and dopamine. Bath temperature 37° C. Every 3 min 0.25 μg histamine added. At N 0.2 μg noradrenaline, at D 20 μg dopamine added to bath. Between arrows 10^{-7} morphine present in bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

in the size of histamine contractions following dopamine less when morphine was present, but the absolute height of contractions after dopamine was larger than in the absence of morphine. After the removal of morphine from the bath, the inhibitory effect of dopamine was fully restored. When the bath temperature was kept at 28° C, the depressant action of dopamine greatly decreased. At 37° C 2×10^{-6} dopamine was more effective than 2×10^{-8} noradrenaline in reducing contractions to histamine. But at 28° C 2×10^{-6} dopamine did not produce any inhibition, and even twice that concentration was less effective than 2×10^{-8} noradrenaline (Fig. 8). Morphine did not affect the reduced response to dopamine at this lower temperature.

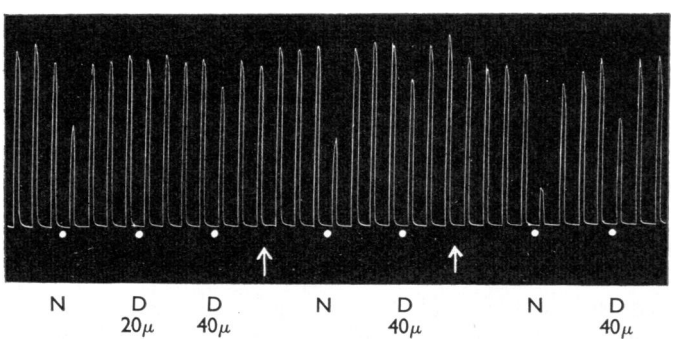

Fig. 6. Effect of morphine on the inhibitory action of noradrenaline and dopamine. Bath temperature 28° C. Every 3 min 0.25 μg histamine added. At N 0.2 μg noradrenaline, at D dopamine in amounts indicated added to bath fluid. Between arrows 10^{-7} morphine present in bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

At 37° C atropine (10^{-8}) did not have any significant effect on the inhibitory action of either dopamine or noradrenaline (Fig. 7). Cocaine 10^{-5} increased somewhat the action of noradrenaline, while leaving that of dopamine unaltered (Fig. 8). Bretylium 10^{-6} did not have any effect on the inhibition produced by any of the amines tested. In all the above experiments the action of ephedrine 10^{-5} was the same as that of noradrenaline.

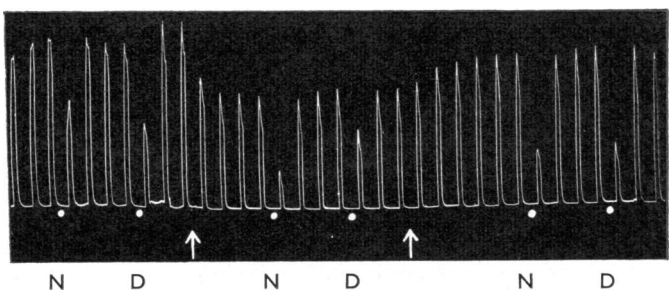

Fig. 7. Effect of atropine on the inhibitory action of noradrenaline and dopamine. Bath temperature 37° C. Every 3 min 0.5 μg histamine added. At N 0.2 μg noradrenaline, at D 20 μg dopamine added to bath. Between arrows 10^{-8} atropine present in the bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

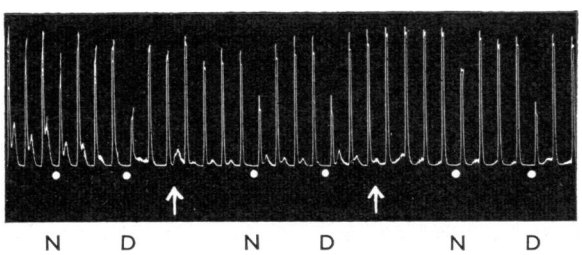

Fig. 8. Effect of cocaine on the inhibitory action of noradrenaline and dopamine. Bath temperature 37° C. Every 3 min 0.4 μg histamine added. At N 0.2 μg noradrenaline, at D 20 μg dopamine added to bath. Between arrows 10^{-5} cocaine present in bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

DISCUSSION

From the observation that bretylium, a drug shown by Boura & Green (1959) to inhibit specifically the release of noradrenaline, abolished the relaxation of the guinea-pig jejunum produced by mesenteric nerve stimulation, it can be concluded that the relaxation was the result of adrenergic nerve stimulation. The concentration and the time required to inhibit the adrenergic nerves of the guinea-pig jejunum were similar to those described by Boura & Green (1959) in the case of the rabbit ileum. The sensitization by cocaine to the response to adrenergic nervous stimulation has been observed in different organs (Trendelenburg, 1959; Hukovic, 1959). The enhancing effect of cocaine on the inhibition produced by

mesenteric nervous stimulation, therefore, also agrees with the conclusion that the relaxation was the result of stimulating adrenergic nerves.

Like bretylium, morphine and atropine were found to decrease the response to the adrenergic stimulation of the jejunum. As it has been shown in the second part of the experimental results, none of these drugs affected the depressant effect of noradrenaline sufficiently to explain this blocking action. The most likely interpretation of these results, therefore, is that the above drugs interfered in some manner with the release of noradrenaline from the nerve endings. The finding that the minimal concentration of morphine that inhibited the adrenergic nerves was about the same as required to reduce the effect of coaxial stimulation on the guinea-pig ileum, as reported by Paton (1957), would indicate that morphine interferes with a step common to cholinergic and adrenergic nerves. This minimal effective concentration is of the same order of magnitude as found in the brain of rats given a small analgesic dose of morphine (Adler, Elliott & George, 1957).

The depressant effect of morphine on the adrenergic nerves could be antagonized partially by nalorphine, whilst, according to Paton (1957), it was impossible to demonstrate conclusively the antagonism of nalorphine against morphine on the cholinergic nerves of the guinea-pig ileum. Although the optimal amount of nalorphine that antagonized the effect of any given concentration of morphine could not be established in this study, it could be clearly shown that nalorphine was effective against morphine present in a much higher concentration. This is in agreement with the *in vivo* findings of Orahovats, Winter & Lehman (1954), who reported that, in the rat, nalorphine antagonized the action of a 16 to 32 times greater dose of morphine. The antagonism of nalorphine to the depressant effect of morphine on the vagus of the rabbit has been also shown by Kosterlitz & Taylor (1959).

Atropine, in a small concentration, decreased the response to adrenergic stimulation without reducing the inhibitory effect of noradrenaline. The slow onset and disappearance of the nervous inhibition by atropine as compared to its antagonisms to acetylcholine suggest that the mechanisms of the two inhibitions are different.

From the observations that the depressant effect of dopamine was reduced when the temperature of the bath was lowered from 37° C to 28° C and that morphine inhibited the depressant action of dopamine only at 37° C, it can be concluded that dopamine acted in different ways at the two temperatures. At 28° C the nervous elements in the intestinal wall are not functioning and only the direct effect of dopamine on the adrenergic receptors could be observed which was not prevented by morphine. On the other hand, at 37° C dopamine acted, in a smaller concentration, on the adrenergic nerve endings, probably by releasing noradrenaline, and this effect was inhibited by morphine. Burn & Rand (1958b) have also shown that dopamine acts both by releasing noradrenaline and also directly on adrenergic receptors on the blood pressure of urethane-anaesthetized guinea-pigs, rabbits and cats.

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