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THE MECHANISM OF THE INHIBITORY ACTION OF ADRENALINE ON THE MAMMARY GLAND

BY

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Antagonism between the actions of adrenaline and oxytocin on the mammary gland was first demonstrated in 1941 by Ely & Petersen. They showed that fright or the intravenous injection of adrenaline reduced the rate of ejection of milk from the udder during machine milking in cows and that this inhibitory effect could be overcome by the injection of oxytocin. Although it is clear that adrenaline inhibits the milk-ejection response to oxytocin in many species (Braude & Mitchell, 1952; Cross, 1953; Whittlestone, 1954; Yokoyama, 1956; Pickford, 1959; Chan, 1965; Bisset, Hilton & Poisner, 1967), the mode of action of adrenaline is uncertain. There are two possible mechanisms. Adrenaline might act indirectly by causing vasoconstriction of the mammary blood vessels, which would limit the access of oxytocin to the gland, or it might have a direct inhibitory effect on the myoepithelial cells and act as a physiological antagonist of oxytocin. Cross (1953; 1954a, b; 1955a, b; 1958) has advanced a number of arguments in favour of the vasoconstrictor hypothesis. In the first place, perfusion experiments have shown that adrenaline is an extremely potent vasoconstrictor of the mammary arteries (Hebb & Linzell, 1951). Secondly it has been observed in the rabbit (Cross, 1953) and in the sow (Whittlestone, 1954) that adrenaline causes greater inhibition when injected before, than simultaneously with oxytocin. This can be explained by supposing that after an injection of adrenaline, the mammary arteries are already constricted when oxytocin reaches the gland in the circulation. Moreover, in the rabbit, Cross & Silver (1961) have made polarographical measurements of oxygen tension (po_2) as an index of capillary blood flow in the mammary gland and shown that, when adrenaline is injected before oxytocin, full inhibition of the milk-ejection response occurs only if oxytocin reaches the gland at a time when the po_2 is falling rapidly. Finally, in the rabbit the relative potencies of adrenaline and noradrenaline are similar for vasoconstriction and inhibition of the milk-ejection response to oxytocin (Cross, 1955a; 1958). Against the argument for a direct inhibitory action on the myoepithelial cells is the fact that adrenaline does not inhibit the milk-ejection response to mechanical tap stimuli applied to the mammary gland (Cross, 1954a, b; 1955a; Yokoyama, 1956) nor does it inhibit contraction of the alveoli when it is applied topically with oxytocin to the living gland of the mouse (Linzell, 1955). On the other hand, adrenaline has been shown to inhibit the action of oxytocin on isolated strips of mammary gland from the rabbit (Chan, 1965; Moore & Zarrow, 1965).

Vasoconstriction would result from an action of adrenaline on α-receptors and, by analogy with the rat uterus (Levy & Tozzi, 1963), a direct inhibitory effect on myoepithelium would be an action on β-receptors. Therefore it should be possible to decide by the use of α- and β-blocking agents which of the two mechanisms of action is involved. Chan (1965) found in the rabbit that the inhibitory effect of adrenaline on the milk-ejection response to oxytocin was not blocked by doses of phenoxybenzamine which reversed its pressor effect. Moreover, angiotensin, in a dose producing the same pressor effect as adrenaline, did not inhibit the response to oxytocin whereas a small dose of isoprenaline, producing a depressor response, did cause inhibition. These results argue against vasoconstriction or an action on α-receptors as the mechanism of action of adrenaline. However, the inhibitory effect of adrenaline was not blocked by a combination of phenoxybenzamine and pronethalol which abolished the vascular response and this argues against an action of adrenaline on β-receptors also.

It seems significant that adrenaline has a pronounced inhibitory effect on the rat uterus, which is due to an action on β-receptors (Levy & Tozzi, 1963) whereas it causes contraction of the rabbit uterus. The existence of such differences between adrenaline receptors in the two species makes it of interest to examine the effect of adrenaline on the milk-ejection response to oxytocin in the rat. A method for assaying milk-ejecting activity in the rat has been described in the preceding paper (Bisset, Clark, Halder, Harris, Lewis & Rocha e Silva, 1967); in this paper it will be shown that adrenaline does inhibit the milk-ejection response to oxytocin in this as in other species and that the inhibition involves both α- and β-receptors. Similar results are described in the guinea-pig.

METHODS

Milk ejection in the rat

Details of the method are described in the preceding paper. A saphenous artery was cannulated for retrograde arterial, and a jugular vein for intravenous, injections. In most experiments a carotid artery was also cannulated and blood pressure recorded using a strain gauge transducer (Statham P23Db) and potentiometric recorder (Leeds Northrup: Speedomax type H).

Milk ejection in the guinea-pig

Milk-ejection pressure was recorded by the method of Bisset (1962). Injections were given *via* the jugular vein or the internal saphenous artery using the technique described by Tindal & Yokoyama (1962). As with the rat, body temperature was maintained at 37° C.

Administration of drugs

Injections of oxytocin were given intravenously or by the retrograde arterial route at intervals of 5 min. The catecholamines were given as a mixture with oxytocin by either route or intravenously 15–60 sec before an intra-arterial injection of oxytocin. The α- and β-blocking drugs were always administered by intravenous injection.

The inhibitory effect of adrenaline

Inhibition of the milk-ejection response to oxytocin by adrenaline was studied in a series of experiments on 14 rats and 4 guinea-pigs. When a dose of adrenaline had been established which produced a substantial inhibition of the milk-ejection response to oxytocin, attempts were made to antagonize the inhibition by injecting the α-receptor blocker phentolamine, and the β-receptor blockers pronethalol, propanolol and Trasicor. The effectiveness of the blockade was demonstrated in several experiments by recording the effect on the blood pressure of large doses of adrenaline given before and after the blocking drugs.

Isolated strips of mammary gland

The method used was that introduced by Méndez-Bauer, Cabot & Caldeyro-Barcia (1960) for the rabbit. A mammary gland was dissected from a lactating rat or guinea-pig and stored overnight at 4° C immersed in Tyrode solution. Radial strips (3 cm×5 mm×5 mm) were cut and suspended in 12.5 ml. Tyrode solution at 37° C. Tension was recorded isometrically using a Statham universal transducing cell (Model UC3) with microscale accessory (Model UL5) coupled with a potentiometric recorder (Goerz, type RE 511). An initial tension of 120–180 mg was applied to the rat gland and 450–1,000 mg to the guinea-pig gland. The tissue was allowed to equilibrate in the organ bath for 1–2 hr before use. Oxytocin was allowed to remain in the bath for 2 min and adrenaline was added simultaneously with or 1 min before oxytocin. The β-receptor blocker Trasicor was added 1 min before adrenaline. Doses were given at intervals of 10–15 min. Measurement of peak tension developed by the tissue proved to be an unreliable assay of effect and did not always permit quantitative analysis. A better parameter was tension-time estimated by means of a simple digital integrator (Lewin, to be published). Such a method takes into account not only the maximum tension achieved but also the latent period and the rate at which tension increases. It was also found that, as with the rat uterus *in situ* (Bisset, Haldar & Lewin, 1966), if the square root of each integrator count was plotted against the logarithm of the dose, a straight line was obtained.

Materials

Drugs used were synthetic oxytocin (Syntocinon, Sandoz); adrenaline hydrogen tartrate (British Drug Houses); *l*-noradrenaline bitartrate (Levophed, Bayer); isoprenaline sulphate (Burroughs Wellcome); phentolamine mesylate (Rogitine, CIBA); pronethalol HCl (Alderlin, I.C.I.); propanolol HCl (Inderal, I.C.I.); 1-isopropyl-amino-2-hydroxy-3-(O-allyloxy-phenoxy)-propane hydrochloride (Trasicor, CIBA). Doses of oxytocin are given in μ-u. (1 μ-u.$=2.2\times10^{-6}$ μg). Weights of the catecholamines are expressed in terms of base and other substances in terms of salt.

All solutions were prepared in 0.9% NaCl solution containing 0.01% ascorbic acid. To enable all doses to be given in a volume of 0.2–0.4 ml., a series of solutions was prepared containing varying concentrations of oxytocin either alone or mixed with the catecholamines.

RESULTS

Rat mammary gland in situ

The inhibitory action of adrenaline

When oxytocin and adrenaline were injected simultaneously by either the retrograde arterial or the intravenous route, adrenaline caused inhibition of the milk-ejection response to oxytocin. This inhibition was transient, since the subsequent injection of oxytocin alone 5 min after adrenaline usually produced a normal response. The inhibitory action of adrenaline was dose dependent. Graded inhibition is shown in Fig. 1. On intra-arterial injection, the threshold dose of adrenaline for inhibiting the response to 40 μ-u. oxytocin was 10 ng; the amount required to produce 50% inhibition was 40 ng. Thus in this experiment the ratio of the dose of adrenaline (ng) to the dose of oxytocin (μ-u.) for 50% inhibition was 1.0. In 9 other experiments in which the dose of oxytocin was 20 μ-u., the ratios were 0.5, 0.25, 0.5, 1.0, 0.25, 0.125, 0.25, 1.0 and 1.0. In 2 experiments with intravenous injections of 200 and 400 μ-u. oxytocin, the ratio for 50% inhibition was 0.25.

Adrenaline also caused inhibition when injected intravenously before oxytocin and the duration of its inhibitory action corresponded with the duration of the pressor response. This was shown in an experiment in which retrograde arterial injections of oxytocin 20 μ-u. were given at various times after adrenaline 200 ng. With intervals of 15, 30 or 60 sec between injections there was the same degree of inhibition, but if oxytocin was

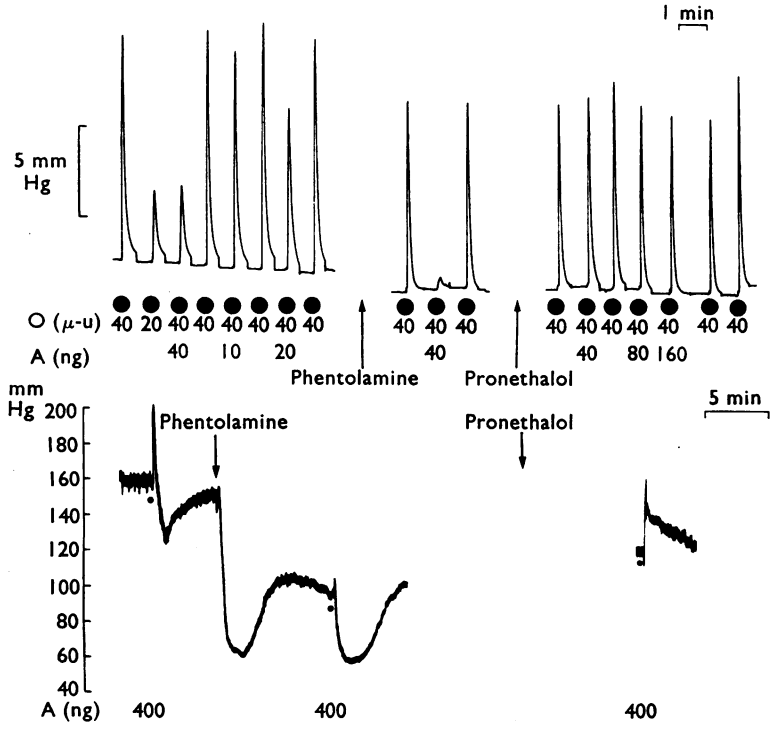

Fig. 1. Lactating rat. Upper record: milk-ejection responses to retrograde arterial injections at 5-min intervals of 20–40 μ-u. oxytocin (O) alone or 40 μ-u. mixed with adrenaline (A). Lower record: arterial blood pressure. Phentolamine (0.4 mg) and pronethalol (2 mg) injected intravenously.

injected 2 min after adrenaline there was only a small inhibition and if 3 min after, no inhibition at all. By this time the pressor response to adrenaline was usually over.

The inhibitory action of noradrenaline and isoprenaline

Not only adrenaline, but noradrenaline and isoprenaline also inhibited the milk-ejection response to oxytocin. Noradrenaline was the least, and isoprenaline the most potent of the three amines. Their potencies were compared by determining the doses required to produce the same inhibition (within the range 50–75%) of the response to a standard amount of oxytocin. When the amines were injected intra-arterially in a mixture with 20 μ-u. oxytocin, the equiactive doses of adrenaline, noradrenaline and isoprenaline were 5, 20 and 2.5 ng, respectively; when intravenous injections were given in a mixture with 200 μ-u. oxytocin (see Fig. 3) the doses were 50, 100 and 25 ng and when the amines were injected intravenously 15 sec before an intra-arterial injection of 20 μ-u. oxytocin the doses were 20, 80 and 20 ng.

The nature of the inhibitory action of adrenaline

(a) Adrenaline injected simultaneously with oxytocin

The inhibitory action of adrenaline, injected with oxytocin by the intravenous or intra-arterial route, could be abolished by β-blocking drugs, but no effect could be obtained

after relatively large doses of drugs blocking α-effects. This was demonstrated in a series of 11 experiments, one of which, using the arterial route, is illustrated in Fig. 1. As described in the previous section, graded inhibition of the response to 40 μ-u. oxytocin was obtained with adrenaline 10–40 ng, 40 ng causing 50% inhibition. After injection of the α-blocker phentolamine (0.4 mg intravenously) the inhibitory effect of adrenaline 40 ng was not reduced. There was no doubt that the dose of phentolamine given was sufficient to cause a block of α-receptors. Although the drug had lowered the blood pressure considerably (lower tracing), intravenous injection of adrenaline 400 ng cause a profound depressor response in contrast to the initial mixed pressor/depressor response. Subsequent injection of the β-blocker pronethalol (2 mg intravenously), however, while reversing the depressor response to adrenaline, abolished the inhibitory action of 40 ng adrenaline and even 160 ng caused a smaller inhibition than that originally obtained with 20 ng.

Not only pronethalol but the β-blockers propranolol and Trasicor also prevented the inhibitory effect of adrenaline and they were considerably more potent. Whereas the threshold dose of pronethalol for abolishing the inhibitory effect of adrenaline was found to be 1 mg, propranolol and Trasicor were effective in doses as small as 10 μg. With 10 μg Trasicor, the block lasted for at least 30 min.

(b) Adrenaline injected before oxytocin

Eight experiments were performed in which adrenaline was injected 15–60 sec before oxytocin. In each case the β-blockers failed to abolish the adrenaline inhibition, a small amount of inhibition always remaining. It was considered possible that under such circumstances part of the adrenaline inhibition might be mediated by an effect on

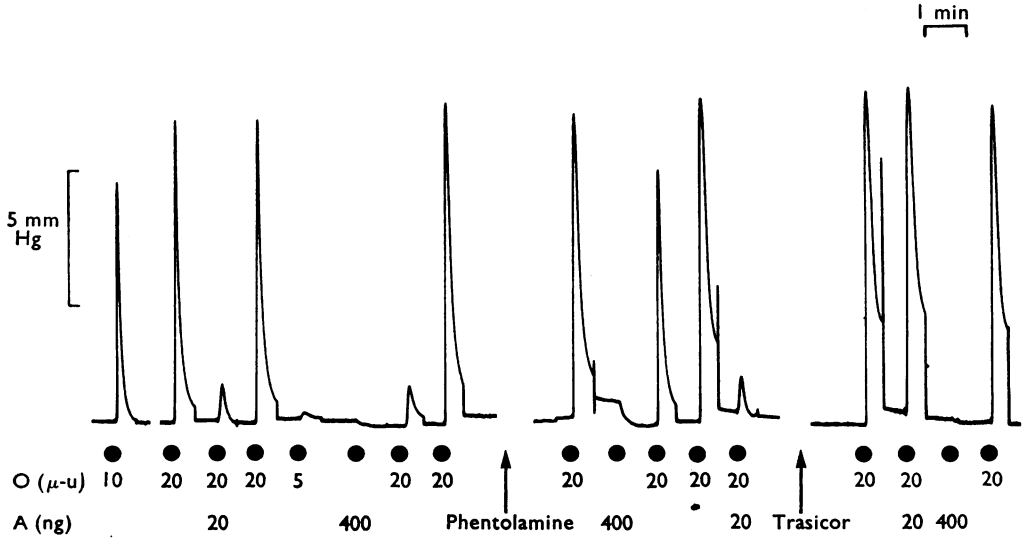

Fig. 2. Lactating rat. Milk-ejection responses to retrograde arterial injections at 5-min intervals of 5–20 μ-u. oxytocin (O) alone, 20 μ-u. mixed with 20 ng adrenaline (A) or 20 μ-u. given 1 min after intravenous injection of 400 ng adrenaline. Phentolamine (0.4 mg) and Trasicor (100 μg) injected intravenously.

α-receptors. The experiment of Fig. 2 shows that this is indeed the case. This experiment compares the effects of the α- and β-blockers on the inhibitory effect of adrenaline given before and simultaneously with oxytocin. Adrenaline 20 ng given intra-arterially with oxytocin inhibited the response of the gland by 75% A similar inhibition was obtained with adrenaline 400 ng given intravenously 1 min before oxytocin. Alpha-blocker phentolamine 0.4 mg caused a marked reduction in the inhibitory action of the large intravenous dose of adrenaline given before oxytocin but did not alter in any way the inhibition caused by the small intra-arterial dose given with the hormone. Trasicor, 100 μg, however, abolished this latter effect and prevented the remaining inhibitory action of the intravenous adrenaline.

The nature of the inhibitory action of noradrenaline and isoprenaline

The question then arose as to whether the inhibition of the responses to oxytocin caused by noradrenaline and isoprenaline, when hormone and catecholamine were given simultaneously, was also an action on β-receptors. The experiment of Fig. 3 shows the inhibition of responses to intravenous injections of oxytocin 200 μ-u. by noradrenaline 100 ng, adrenaline 50 ng and isoprenaline 25 ng. The injection of Trasicor 10 μg abolished or practically abolished the inhibitory action of all three amines, whereas, although not illustrated, phentolamine 0.4 mg had had no effect.

Guinea-pig mammary gland in situ

The observations made on the mechanism of action of adrenaline on the mammary gland of the rat were found to apply also to the guinea-pig.

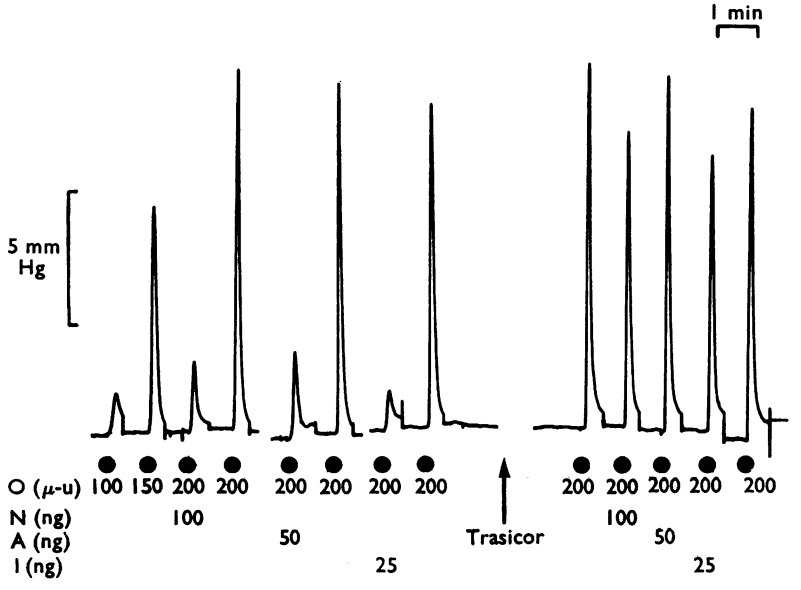

Fig. 3. Lactating rat. Milk-ejection responses to intravenous injections at 5-min intervals of 100–200 μ-u. oxytocin (O) alone or 200 μ-u. mixed with 100 ng noradrenaline (N), 50 ng adrenaline (A) or 25 ng isoprenaline (I). Trasicor (10 μg) injected intravenously.

In 4 experiments in which adrenaline and oxytocin were injected simultaneously by the retrograde arterial route, 10–50 ng adrenaline was required to inhibit by 50% the milk-ejection responses to oxytocin 200–400 μ-u. This action was abolished by Trasicor (100–800 μg) and was unaffected by phentolamine.

However, as in the rat, the inhibition of the response to oxytocin produced by intravenous injection of adrenaline 50–200 ng before oxytocin was not completely prevented by injecting Trasicor alone, but was abolished by a combination of Trasicor and phentolamine.

Isolated strips of mammary gland

The responses to oxytocin of isolated strips of mammary gland from the rat and guinea-pig were inhibited by adrenaline. The ratio of the dose of adrenaline (μg) to the dose of oxytocin (m-u.) for 50–75% inhibition was determined in each experiment. For the rat, with doses of oxytocin from 2–8 m-u., the ratios were: 0.5, 1, 1, 1, 0.5, 0.25, 0.25 and 0.05 and, for the guinea-pig, with doses from 1–10 m-u., 1, 0.5, 0.25 and 2. In some experiments, although intervals of 10–15 min were allowed between additions of drugs to the organ bath, tachyphylaxis to the inhibitory effect of adrenaline was observed. Often, too, there was a prolonged inhibition of the response to oxytocin after repeated doses of adrenaline. This desensitization made it difficult to assess quantitatively the inhibition caused by adrenaline.

The inhibitory effect of adrenaline was blocked by Trasicor. An experiment which was not complicated by desensitization, is illustrated in Fig. 4. When 2 μg adrenaline was added to the bath 1 min before 4 m-u. oxytocin, the response was reduced by 75% —that is, it was equivalent to the response previously produced by 1 m-u. oxytocin. This effect was repeated, but when adrenaline was added in the presence of 10 μg

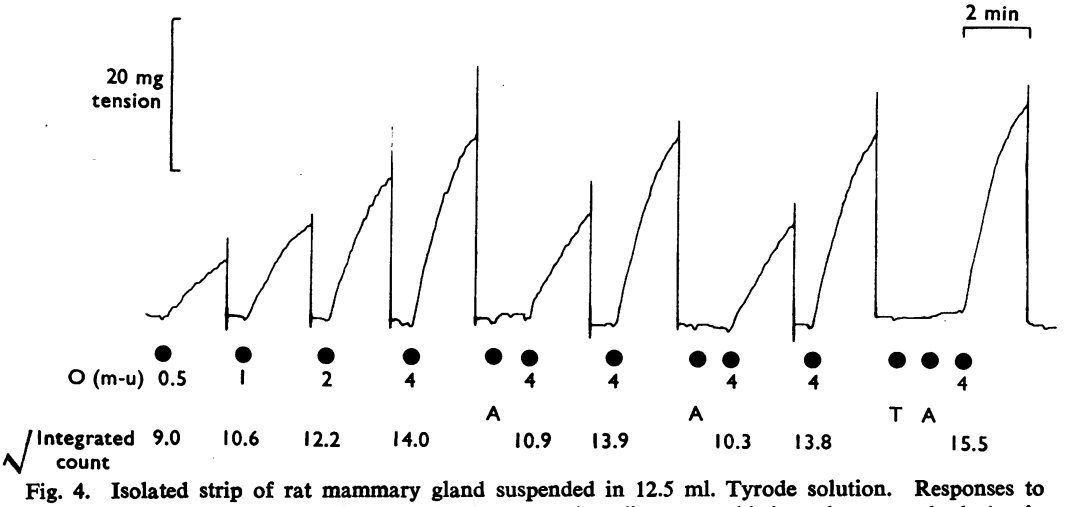

Fig. 4. Isolated strip of rat mammary gland suspended in 12.5 ml. Tyrode solution. Responses to 0.5, 1, 2 or 4 m-u. oxytocin (O). At A, 2 μg adrenaline was added to the organ bath 1 min before oxytocin and at T, 10 μg Trasicor was added 1 min before adrenaline. The organ bath was washed out 2 min after each addition of oxytocin, but not after adrenaline or Trasicor. An integrator was used to estimate tension-time and the numbers in the bottom row of the figure give the square roots of the 2-min counts (see Methods).

Trasicor the inhibition was abolished. In some experiments, smaller doses of Trasicor were effective and, with the most sensitive preparation, as little as 2 μg prevented the inhibitory action of 2 μg adrenaline. The effect of Trasicor was long lasting. In some cases the inhibitory effect of adrenaline did not return within the duration of the experiment. However in 3 experiments the inhibitory effect of Trasicor was completely restored after intervals ranging from 20 to 120 min, and in 3 other experiments partially restored in 10–60 min. Restoration of the response to adrenaline in these experiments excludes the possibility that the blocking effect of Trasicor was only apparent, and in reality due to desensitization of the tissue to adrenaline.

DISCUSSION

There seems little doubt that the inhibitory effect of small doses of adrenaline injected simultaneously with oxytocin in the rat or guinea-pig is mediated wholly through an action on β-receptors. Since adrenaline is one of the substances which is likely to be present in blood extracts and to interfere with the assay of oxytocin by the method described in the preceding paper, the results of the present experiments provide a method of eliminating this source of interference by previous administration of a β-receptor blocker.

The inhibition of milk-ejection responses to oxytocin was abolished by β-receptor blockers but not affected by doses of α-receptor blockers large enough to reverse the pressor response to adrenaline. The effect of adrenaline was antagonized by three different β-blockers and, in agreement with their relative potencies in antagonizing β-effects in the cardiovascular system (Brunner, Hedwall & Meier, 1967), propanalol and Trasicor were much more potent than pronethalol. Like adrenaline, noradrenaline and isoprenaline caused inhibition of the milk-ejection response to oxytocin, and the order of potency was the same as that observed in the rabbit by Chan (1965)—that is, isoprenaline was the most, and noradrenaline the least, potent inhibitor. This is consistent with the order of potency which is generally accepted for β-receptor stimulating activity.

Further evidence for the presence in the mammary gland of β-receptors is provided by the fact that adrenaline acted upon isolated strips in which there was no possibility of its inhibitory effect being explained by an action on the α-vasoconstrictor receptors of blood vessels. Moreover, the inhibitory effect of adrenaline on isolated strips was blocked by Trasicor and, in contrast with Chan's findings in the rabbit the dose ratio of adrenaline and oxytocin for producing a given amount of inhibition in the isolated rat gland and the gland *in situ* was similar. Oxytocin produces milk-ejection by causing contraction of the myoepithelial cells surrounding the alveoli and smaller ducts in the mammary gland (Richardson, 1947, 1949; Linzell, 1952, 1955, 1961). These cells are the most probable site of β-receptors for adrenaline.

Under certain conditions, it seems that a small part of the inhibitory effect of adrenaline on the mammary gland of the rat and guinea-pig is the result of an action on the α-receptors of blood vessels. This occurs when adrenaline is injected before oxytocin. In these experiments β-blockers, even in high doses, did not completely prevent the action of adrenaline and an inhibitory effect still remained which could be abolished by an α-blocker. In these circumstances it is reasonable to assume, in accordance with Cross's view, that vasoconstriction has occurred before oxytocin is injected, thus delaying access

of the hormone to the gland. It seems likely that the discrepancy between our results in the rat and those obtained in the rabbit by Chan (1965) is due to species differences.

SUMMARY

1. Adrenaline inhibited the milk-ejection response to oxytocin in the lactating rat and guinea-pig.

2. When adrenaline was injected simultaneously with oxytocin, its inhibitory effect was not reduced by doses of the α-blocking agent phentolamine sufficient to prevent vasoconstriction but it was abolished by the agents blocking β-receptors, pronethalol, propanolol and Trasicor.

3. When adrenaline was injected before oxytocin, inhibition was blocked only partly by Trasicor but completely by a combination of Trasicor and phentolamine.

4. Adrenaline also inhibited the action of oxytocin on isolated strips of mammary gland from the rat and guinea-pig and the inhibition was blocked by Trasicor.

5. It is concluded that there are β-receptors for adrenaline in the mammary gland directly mediating the inhibitory action of adrenaline. These β-receptors, which are extravascular, are most likely to be situated in the myoepithelial cells. The inhibition which occurs when adrenaline is injected before oxytocin involves an action not only on β-receptors but also on α-receptors probably in the mammary blood vessels.

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