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RECEPTORS FOR CATECHOL AMINES IN THE SUBMAXILLARY GLANDS OF RATS

BY

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The conception of α- and β-receptors for catechol amines was originally based on experiments with heart and smooth muscle (Ahlquist, 1948). The present investigation deals with such receptors in glandular tissue. The starting-point was the observation that guanethidine causes a flow of saliva from the submaxillary gland which is completely abolished by dihydroergotamine in cats but only reduced by this drug in rats; further experiments disclosed that this applies to secretion evoked by noradrenaline also (Emmelin & Strömblad, 1963). It may therefore be assumed that the rat salivary gland is supplied not only with α-, but also with β-receptors. This conclusion is supported by the fact that saliva flows from the mouth of rats after intraperitoneal injection of isoprenaline (Selye, Veilleux & Cantin, 1961; Argonz, 1962; Wells, 1962; Pohto & Paasonen, 1964); a salivary flow has also been observed from the submaxillary duct of rats after large doses of isoprenaline given intraperitoneally (Schneyer, 1962; Ohlin, unpublished) or small intravenous doses (Ohlin, 1964).

METHODS

Fifty rats weighing between 180 and 480 g were used. During preliminary ether anaesthesia a femoral vein was cannulated using a fine polyethylene tube, through which chloralose (100 mg/kg) was given. In the course of the experiment, which lasted for many hours, the anaesthesia sometimes became superficial, and pentobarbitone, 20 mg/kg, was then injected intraperitoneally. A tracheal cannula was inserted and fine glass cannulae were introduced into the two submaxillary ducts, exposed in the neck. Cannulation of the femoral vein and the salivary ducts was easier when a dissection microscope was used.

Drops of saliva (about 10 μl. in volume), falling from the cannulae, were signalled on a smoked drum using an electromagnetic signal operated manually. The sympathetic trunk was exposed in the neck and stimulated electrically for periods of 1 min at a rate of 20 shocks/sec. The following drugs were given intravenously: methacholine, adrenaline, isoprenaline, dihydroergotamine, phenoxybenzamine, dichloro-isoprenaline, pronethalol hydrochloride and atropine. In an attempt to increase the secretory responses by sensitizing the gland, the right chorda-lingual nerve was frequently cut, usually 2 to 4 weeks before the acute experiment. In two rats the right superior cervical ganglion was instead removed in advance (33 to 44 days). These operations were made during ether anaesthesia.

RESULTS

At the start of an experiment there was usually no flow of saliva from the submaxillary glands, but occasionally a small secretion was seen. Sometimes it might have been caused by a sympathetic hyperactivity due to the operative trauma or the ether anaesthesia; it decreased in rate and soon ceased and it could be stopped by deepening the anaesthesia

by means of pentobarbitone, or diminished by injecting phenoxybenzamine. In two experiments, however, it continued throughout the experiment and was not affected by these drugs, or by atropine or by removal of the superior cervical ganglion and the adrenal medullae. These exceptional glands might have contained cells endowed with the ability to secrete " spontaneously " (Babkin, 1950), as regularly seen in the sublingual gland of the cat (Emmelin, 1953) and the submaxillary gland of the rabbit (Nordenfelt & Ohlin, 1957); anatomical control at the end of these experiments showed that the cannulae had not been inserted by mistake in the sublingual ducts.

Small secretory responses of an average size of one drop could be evoked in normally innervated glands by injecting intravenously methacholine (5 μg/kg) or adrenaline (20 μg/kg). After isoprenaline (10 μg/kg) about half a drop was obtained. Previous section of the parasympathetic nerves to the glands increased the effects of these doses, with methacholine and isoprenaline by about 50% and with adrenaline by nearly 200% (Table 1). Removal of

TABLE 1

EFFECT OF PREVIOUS SECTION OF THE CHORDA-LINGUAL NERVE ON THE RESPONSES TO METHACHOLINE (5 μG/KG), ADRENALINE (20 μG/KG) AND ISOPRENALINE (10 μG/KG)

The figures are mean numbers of drops, obtained from twenty-three rats in the case of methacholine and adrenaline, and from eighteen of these rats for isoprenaline

Condition of gland	Salivary flow (drops) after		
	Methacholine	Adrenaline	Isoprenaline
Innervated	1·03	1·07	0·59
Denervated	1·52	2·80	0·95

the sympathetic ganglion caused some supersensitivity also. Usually the three drugs were given in succession at intervals of 5 min. In one and the same experiment the responses remained remarkably constant. In one control experiment, for instance, the drugs were given over a period of more than 5 hr, and the responses were practically the same throughout the experiment; pentobarbitone, which had to be given intraperitoneally in this experiment, did not change the secretory responses. Sympathetic stimulation for 1 min usually evoked a secretion of 1 to 3 drops from the normally innervated glands.

Dihydroergotamine and phenoxybenzamine. When dihydroergotamine (0.1 mg/kg) or phenoxybenzamine (0.5 to 1 mg/kg) was given intravenously the response to adrenaline was greatly reduced, but usually not abolished; instead of one drop, for instance, about a quarter of a drop or less was obtained. The responses to methacholine and isoprenaline were not affected. The antiadrenaline effect reached its maximum a few minutes after injection of dihydroergotamine but not until 15 to 30 min after injection of phenoxybenzamine. The gland, sensitized by section of the chorda-lingual nerve or removal of the sympathetic ganglion, still gave a larger response to adrenaline than the contralateral gland. In some experiments, when the initial secretory effect of adrenaline on the normal gland had been small, no effect at all was seen when the blocking agent had been given; usually, however, at least a trace of saliva appeared after adrenaline, and this was a true secretory effect and not an expulsion of saliva present in the ducts, as indicated by the fact that repeated doses of adrenaline caused equal effects. The antiadrenaline effect was of long duration, lasting for at least 2 hr. It was not further increased by raising the dose of the blocking agent. Thus, the dose of dihydroergotamine was often gradually increased to a total of 2 mg/kg and that of phenoxybenzamine to 6 mg/kg. At these levels the responses

to methacholine and isoprenaline remained unchanged. It was noticed, however, that, if the initial dose of dihydroergotamine were unnecessarily high, for instance 0.5 mg/kg, the effect of methacholine could be temporarily depressed and that to adrenaline even abolished for a period of about 30 min; the routine procedure was therefore to raise the dose gradually, until no further blocking effect was obtained.

The secretory responses to sympathetic stimulation were reduced but not abolished by dihydroergotamine and phenoxybenzamine. The doses required for maximal blocking effect were somewhat larger than those for adrenaline, about 0.3 mg/kg of dihydroergotamine and 5 mg/kg of phenoxybenzamine; and the reduction of the secretory responses was not as pronounced as that obtained when adrenaline was used to evoke secretion.

TABLE 2

EFFECTS OF METHACHOLINE (5 μG/KG), ADRENALINE (20 μG/KG), ISOPRENALINE (10 μG/KG) AND SYMPATHETIC STIMULATION (FOR 1 MIN) BEFORE AND AFTER DIHYDROERGOTAMINE OR PHENOXYBENZAMINE

The doses of dihydroergotamine or phenoxybenzamine had been gradually raised until no further reduction of the response to adrenaline or sympathetic stimulation was obtained (see text). The figures represent mean numbers of drops; secretion was elicited by drugs in eleven rats and by sympathetic stimulation in seven other rats. In the denervated glands the chorda-lingual nerve had been cut previously

	Salivary flow (drops) in denervated gland after			Salivary flow (drops) in innervated gland after			
Condition	Metha-choline	Adren-aline	Isopren-aline	Metha-choline	Adren-aline	Isopren-aline	Sympathetic stimulation
Before blocking drug	1·49	2·30	0·92	1·12	1·13	0·66	2·73
After blocking drug	1·28	0·21	0·82	1·02	0·08	0·61	0·78

Table 2 shows that dihydroergotamine and phenoxybenzamine had little effect on the responses to methacholine and isoprenaline but greatly diminished those to adrenaline and sympathetic stimulation without, however, abolishing them.

Dichloroisoprenaline. The dominating effect of dichloroisoprenaline was to cause a flow of saliva. Whereas 10 μg/kg had no effect, 100 μg/kg evoked a slow and very long-lasting secretion, greater on the side where the chorda-lingual nerve had been cut in advance; it was seen to continue for more than 5 to 6 hr. The flow increased with the dose. In one experiment, for instance, it amounted to one drop in 3 min on the sensitized, and one in 7 min on the control side after 1 mg/kg. As much as 5 mg/kg was given in some rats, still causing a rapid secretion. The secretory responses were not reduced by dihydroergotamine or phenoxybenzamine, even in high dosage. Thus secretion, elicited by 100 μg/kg of dichloroisoprenaline was in one instance not affected by a total dose of 1.9 mg/kg of dihydroergotamine and 5 mg/kg of phenoxybenzamine. Because of the secretory activity of the drug it was difficult to estimate whether it had any antagonistic effect against isoprenaline. In some experiments, when the secretion was slowing down several hours after an injection of dichloroisoprenaline, the responses to isoprenaline seemed somewhat reduced, whereas the effects of methacholine and adrenaline were higher than when no secretion was going on.

Pronethalol. The rats often tolerated large doses of this drug provided that a small dose (2 mg/kg) was first given and the amount then gradually raised after intervals of 15 to 30 min. In many experiments 20 to 30 mg/kg could be given in this way, and sometimes 50 mg/kg or more.

Pronethalol was found to have a secretory action, but it was much less pronounced than that due to dichloroisoprenaline, and very variable. Sometimes no secretion was observed even after large doses. Sometimes the administration of about 2 mg/kg started a very slow secretion; additional doses accelerated the flow in some rats, but often they had little effect. The highest rate of secretion was one drop in 5 min from a normal gland, after a total dose of 37 mg/kg of pronethalol; usually, however, the flow was much slower and often just perceptible. When the chorda-lingual nerve had been cut or the sympathetic ganglion removed in advance the secretion was more rapid than on the control side. Characteristically the flow started after some minutes' delay, increased slowly and went on for hours. It was not affected by atropine (1 mg/kg), phenoxybenzamine (10 mg/kg) or dihydroergotamine (highest dose, 16.3 mg/kg).

The secretory effect of methacholine was never diminished by pronethalol; sometimes it was increased, when pronethalol itself caused secretion. An antagonistic action against isoprenaline could usually be demonstrated when the dose of pronethalol had been raised to 5 to 10 mg/kg. This effect, which was best seen when pronethalol caused little or no secretion, increased with the dose of pronethalol. A dose of pronethalol which completely abolished the secretory action of isoprenaline was, however, never reached. Thus, in one experiment isoprenaline (10 μg/kg) caused a flow of nearly one drop of saliva after 5 mg/kg of phenoxybenzamine; successive doses of pronethalol reduced this effect gradually but, even after 52 mg/kg, isoprenaline still produced a small trace of saliva and an additional dose of pronethalol was lethal. When pronethalol caused secretion, the response to adrenaline was often slightly increased, but in other experiments the effect of adrenaline was reduced, and in some instances the small secretory response to adrenaline remaining after dihydroergotamine or phenoxybenzamine was completely abolished by pronethalol.

DISCUSSION

The experiments suggest that the concept of α- and β-receptors for catechol amines can be applied to the rat submaxillary gland. Adrenaline and isoprenaline cause secretion, and drugs described as α-blocking agents, dihydroergotamine and phenoxybenzamine, reduce the secretory response to adrenaline but do not affect that to isoprenaline. As in other tissues, isoprenaline therefore seems to react with β-receptors, and adrenaline with both types of receptors and particularly with those of the α-type, since the α-blocking drugs very much diminish the secretory effect of adrenaline. Similarly, the secretory responses to sympathetic stimulation are much diminished but not entirely abolished by the α-blocking agents and, characteristically, larger doses of these drugs are needed when sympathin is released from the nerve endings in close contact with the gland cells than when adrenaline reaches these cells by way of the blood.

Of the so-called β-blocking agents, dichloroisoprenaline has such a pronounced secretory activity that a blocking effect is difficult to detect. The stimulating effect of the drug seems to be exerted via the β-receptors since it is not antagonized by dihydroergotamine or phenoxybenzamine. The dominating effect of dichloroisoprenaline on the gland is thus the sympathomimetic one, originally found in the heart by Dresel (1960).

Pronethalol is more promising as a β-blocking compound in the gland than is dichloroisoprenaline. The drug has some stimulating effect on the β-receptors, but this effect is much smaller than that caused by dichloroisoprenaline, and a definite β-blocking component

can often be demonstrated when either isoprenaline or adrenaline is used to evoke secretion. The effects of pronethalol on the gland are apparently similar to those on heart and smooth muscle (Black & Stephenson, 1962; Donald, Kvale & Shepherd, 1964).

From the observations of the present study it may also be concluded that supersensitivity after cutting the parasympathetic nerves to the gland or sympathetic denervation applies both to α- and β-receptors.

SUMMARY

1. Experiments were carried out on rats anaesthetized with chloralose to investigate whether both α- and β-receptors for catechol amines are present in the submaxillary gland. Salivary secretion was evoked by intravenous injection of adrenaline, methacholine and isoprenaline, or by sympathetic stimulation. To increase the responses the chorda-lingual nerve was often first cut on one side or the superior cervical ganglion was removed.

2. Secretion caused by adrenaline or sympathetic stimulation was greatly reduced but usually not abolished by dihydroergotamine or phenoxybenzamine. The responses to methacholine and isoprenaline were not affected.

3. The secretory effect of adrenaline persisting after these blocking agents could be further diminished or even abolished by pronethalol. Pronethalol often had some secretory effect. The dominating effect of dichloroisoprenaline was to cause secretion.

4. It is concluded that the rat submaxillary gland contains both α- and β-receptors for catechol amines. Both types of receptors can be sensitized by previous section of the chorda-lingual nerve or by removal of the superior cervical ganglion.

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