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THE EFFECT OF CATECHOLAMINES ON UNIT ACTIVITY IN AFFERENT NERVES FROM THE ADRENAL GLANDS

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SUMMARY

1. Afferent discharges were observed in dissected filaments of nerves to the adrenal gland in the rabbit and cat.

2. Systemic intravenous and close intra-arterial injections of (−)-adrenaline and (−)-noradrenaline caused cessation or depression of the spontaneous firing rate.

3. Intravenous injection of acetylcholine and electrical stimulation of the splanchnic nerve also depressed the afferent discharge rate.

4. Modification of blood pressure levels by chemical and mechanical means did not modify the afferent discharge.

5. Alpha blocking agents (ergotamine and phenoxybenzamine) blocked the effect of (−)-adrenaline on the firing rate. Beta blocking agents (dichloroisoprenaline and propranolol) were without effect.

6. It is suggested that an afferent system is present in the adrenal gland. Within this system are chemosensitive receptors which may constitute the afferent limb of a local feed-back loop involved in adrenal catecholamine release.

INTRODUCTION

Efferent innervation of the adrenal gland is a well established fact. The existence of an afferent innervation, however, has not been established, nor has it seriously been suggested. Afferent systems have been described for many other visceral structures. The reviews by Downman (1963) and Paintal (1963) clearly point out the wide variety of sensory receptors present in the viscera and the ways in which these afferents participate in reflex activities. Downman (1955) has also shown specific involvement of the splanchnic nerve (the major source of adrenal fibres) in these activities.

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The results of these studies and others (see Discussion for references) which show that catecholamine administration can modify neuronal firing rate, suggested that an afferent system might be present in the adrenal gland and, moreover, that such a system might be sensitive to catecholamine levels. The evidence to support such an hypothesis is presented below. Preliminary findings have appeared elsewhere (Niijima & Winter, 1967).

METHODS

Experiments were conducted on forty rabbits and twenty-four cats. Adult animals of both sexes were used. The animals were anaesthetized with sodium pentobarbitone 30 mg/kg injected intraperitoneally. Rectal temperatures were kept between 36·5 and 39° C by a heating pad. Respiration was monitored through one arm of a T-tube cannula inserted into the animal's trachea. Arterial blood pressure was recorded from polyethylene catheters placed in the cardiac end of the common carotid artery and/or in the caudal portion of the abdominal aorta. Drugs were injected through a catheter in the jugular vein or, in some instances, through a catheter placed in the abdominal aorta at the level of the renal arteries. The drugs were (−)-adrenaline, U.S.P. (Vitarine); (−)-noradrenaline, U.S.P. (Winthrop); isoprenaline (isoproterenol hydrochloride, Vitarine); ergotamine tartrate (Sandoz); acetylcholine chloride (Merck); phenoxybenzamine hydrochloride (S.K.F.); dichloroisoprenaline (dichloroisoprotenenol hydrochloride, Ayerest); and propranolol hydrochloride (Ayerest).

The small nerves to the adrenal gland were located and isolated, and all branches which did not lead directly to the gland were cut. Recordings were taken from the peripheral portions of these nerves after their central connexions were severed. Unit discharges were recorded through a negative-capacitance electrometer and stored on magnetic tape. All analyses of unit activity took place after conversion of the raw data to standard pulses by a sensitive window discriminator. The standard pulses were then led to a small digital computer for further analysis. A description of this system appears elsewhere (Caggiano, Hall & Winter, 1966).

On-line analysis of afferent discharge (total events per unit time) was recorded on a polygraph along with blood pressure, e.c.g. cardiotachograph and respiratory excursions. Detailed analyses of single afferents were performed at a later time.

RESULTS

Nature of the physiological stimulus. When a small filament was dissected from a nerve branch to the adrenal gland and placed on the recording electrode, spontaneous afferent discharges were often observed. A small amount of (−)-adrenaline was injected intravenously in an attempt to modify the unit activity. Some units showed an increase in discharge rate accompanied by an increase in blood pressure. Evidence will be presented elsewhere that these units are baroreceptors (A. Niijima & D. L. Winter, to be published). They will not be discussed in this report. Other units showed a decrease in firing rate after the injection of adrenaline. The behaviour of these units constitutes the basis of this study. These findings were consistently observed in both the cat and rabbit. No species differences

were noted. The large number of animals used reflected the difficulty of the nerve dissection procedure.

Figure 1 shows the effect of an intravenous injection of 20 μg of (−)-adrenaline, onset indicated by the arrow, on one such unit. During the control period (top line) some rhythmicity in firing rate was noticeable which was not related to respiration. Following the (−)-adrenaline injection, the firing decreased and stopped, in this case rather abruptly, and then gradually returned to control levels. Cessation of firing lasted about 25 sec. A transient increase in blood pressure was also observed which slowly returned to control level.

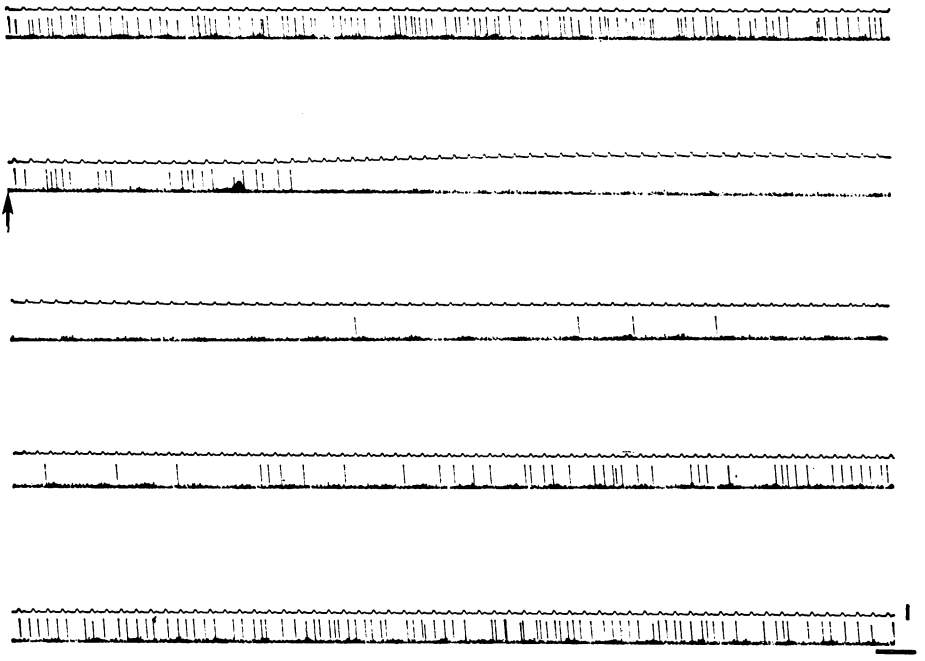

Fig. 1. The effect of (−)-adrenaline on the afferent discharge rate. Rabbit. Upper trace in each pair of records represents the blood pressure curve and lower trace the unitary afferent discharges recorded from a nerve filament to the adrenal gland. All records are continuous. The vertical bar at the bottom represents the blood pressure level from 0 to 200 mm Hg. The horizontal bar represents 1 sec. The control recording at the top shows that the receptor has tonic activity. At the arrow, 20 μg (−)-adrenaline was injected intravenously.

(−)-Noradrenaline was also effective in reducing the firing rate of these units. Figure 2*A* shows the effects of intravenous injections of (−)-adrenaline 20 μg (left) and (−)-noradrenaline 20 μg (right). In this and the following figures, unit activity is represented by the computer output

which shows a running mean value. The number of spikes that occurred each second is successively displayed. The vertical calibration bar to the right represents 0 (bottom) and 10 (top) spikes/sec.

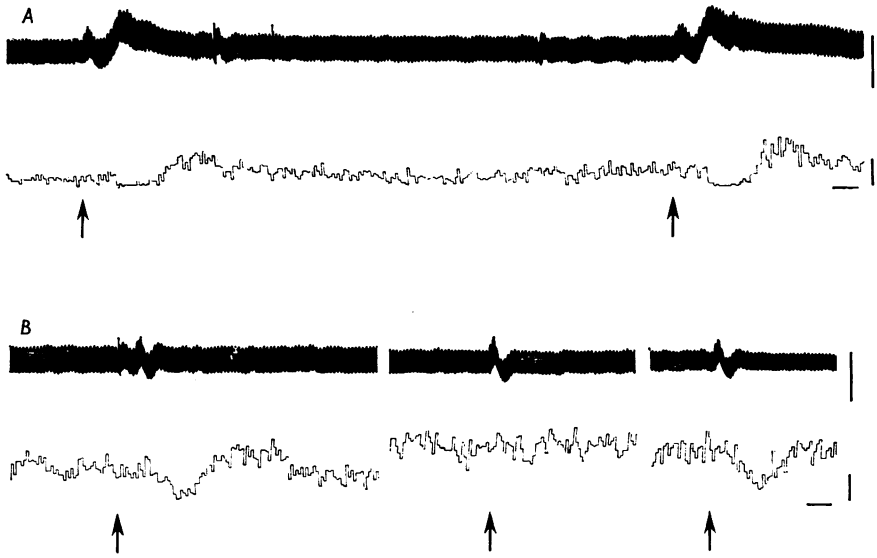

Fig. 2. Effect of small doses of (−)-adrenaline and (−)-noradrenaline on the afferent discharge rate. Rabbit. *A*. Intravenous injection of 20 μg (−)- adrenaline (left) and (−)-noradrenaline (right). *B*. Intravenous injection of 1 μg (−)-adrenaline (left) and (−)-noradrenaline (right). Middle arrow shows injection of physiological saline (2 ml.). Calibrations in this and subsequent figures: Vertical bars (blood pressure) 0–100 mm Hg., (unit activity) 0 to 10 spikes/sec. Horizontal bars: 10 sec.

It was possible in some cases to show an effect following the injection of as little as 1 μg of test solution. Figure 2*B* shows the modification of afferent firing rate following 1 μg (−)-adrenaline (left) and 1 μg (−)-noradrenaline (right). Two millilitres of physiological saline (0·9 g/100 ml.) (centre) produced no effect. The comparable blood pressure elevation following the saline injection suggests that the pressure change is not an adequate stimulus. This experiment was from the same animal as shown in Fig. 2*A* but a different nerve preparation. The response to such small doses of test drugs was often difficult to demonstrate and in this record, only, a multiunit preparation was used. All other records are from single unit preparations.

Close arterial injections were made from a catheter in the abdominal aorta which extended to the level of the adrenal arteries. In these experiments drug administrations had a more remarkable and prolonged effect. The latency of this effect on afferent discharge rate was shortened.

Additional experiments were performed to determine the relationship between afferent firing rate and blood pressure levels. Figure 3*A* shows the effects of intravenous injections of 20 μg (−)-adrenaline (left) and 10 mg acetylcholine (right). In both experiments there was a decrease in firing rate although the blood pressure increased and decreased respectively. In Fig. 3*B*, 75 μg of isoprenaline (left) caused a decrease in blood pressure with no change in afferent discharge, while 20 μg (−)-adrenaline (right)

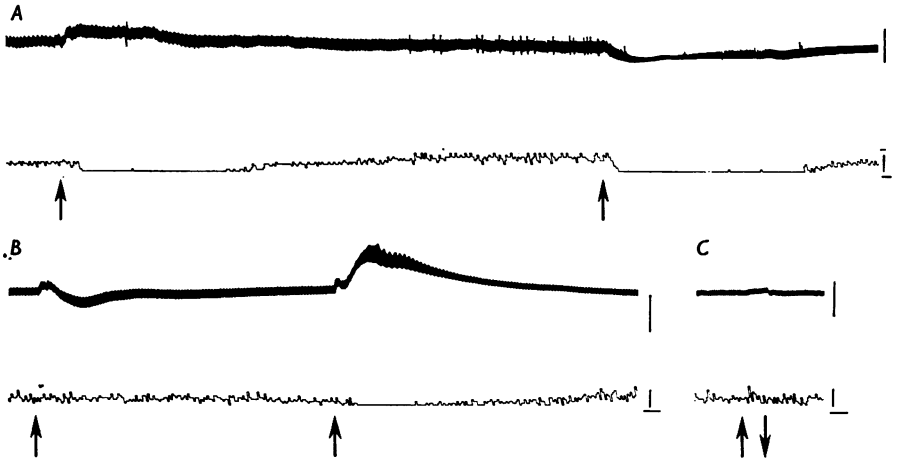

Fig. 3. Effects of blood pressure changes induced by chemical and mechanical means on afferent discharge rate. Cat. *A*. Intravenous injection of 20 μg (−)-adrenaline (left) and 10 mg acetylcholine (right). *B*. Intravenous injection of 75 μg isoprenaline (left) and 20 μg (−)-adrenaline (right). *C*. Clamp of abdominal aorta below level of adrenal artery.

evoked the typical response seen above. Figure 3*C* shows the effect of clamping the abdominal aorta below the level of the adrenal arteries. No change in firing rate was noted. A large injection of saline and clamping the abdominal aorta above the level of the adrenal arteries (Fig. 4*A* and *B*) also evoked little change in afferent discharge. Unlike other units in the adrenal afferent nerves (A. Niijima & D. L. Winter, to be published), these units seem to respond independently of the blood pressure level. The common feature of these units is that their firing rate seems to reflect conditions in which adrenaline or noradrenaline content in the circulation of the adrenal gland is modified. It is well known that the application of acetylcholine to the adrenal gland increases the release of adrenaline and noradrenaline. We interpret the record in Fig. 3*A* (right) in this light.

Another condition which causes the release of adrenaline from the adrenal gland is electrical stimulation of the splanchnic nerve. Figure 5 demonstrates that afferent discharge decreases following such stimulation.

In the top line, an injection of 10 μg (−)-adrenaline (left) is followed by electrical stimulation at 15 c/s (right). In the bottom line, in another unit, 20 μg (−)-adrenaline (left) is followed by electrical stimulation at 30 c/s. (right). In both cases, the afferent discharge rate was clearly depressed following electrical stimulation. Also, the effect could be graded by varying the stimulation parameters.

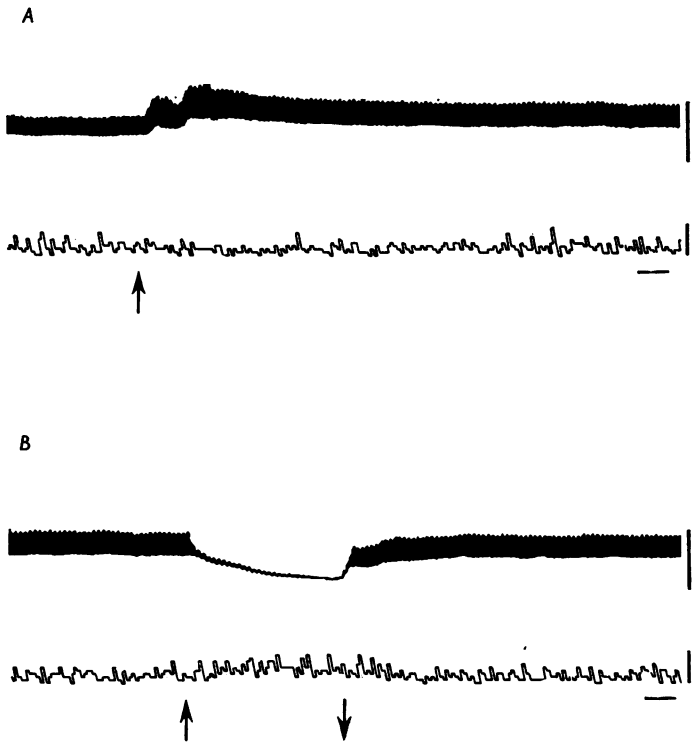

Fig. 4. Effect of mechanically induced increase and decrease of blood pressure on afferent firing rate. Cat. *A*. Intravenous injection of 20 ml. saline. *B*. Clamp of abdominal aorta above level of adrenal artery.

Effects of pharmacological blocking agents. Alpha and beta blocking agents were tested in a subsequent series of experiments. In Fig. 6, the alpha blocking agents ergotamine tartrate and phenoxybenzamine were used. Record *A* is the control response to 10 μg (−)-adrenaline. Ten minutes after the injection of 1 mg ergotamine, a test injection of 10 μg (−)-adrenaline was not effective in depressing the discharge rate (record *B*). Similar effects were seen following phenoxybenzamine administration. Record *C* is the control response to 10 μg (−)-adrenaline and *D* represents the response to 10 μg (−)-adrenaline 40 min after phenoxybenzamine

(50 mg) had been injected. In both cases the depressive effect of adrenaline injection on the discharge rate had been blocked.

We were unable to observe any effect, however, following the administration of beta blocking agents. Figure 7 demonstrates the ineffectiveness of 15 mg dichloroisoprenaline (DCI) (control *A*, test *B*) and 5 mg propranolol (control *C*, test *D*) on firing rate following the injection of 10 μg

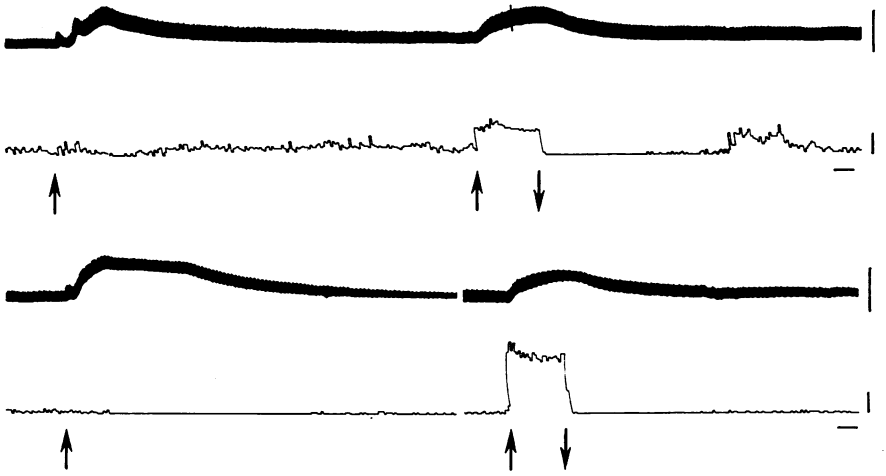

Fig. 5. Comparison of effect of electrical stimulation of splanchnic nerve and injection of (−)-adrenaline on afferent discharge rate. Cat. Single arrow indicates the time of intravenous (−)-adrenaline injection (10 μg). A pair of arrows indicates the onset and end of electrical stimulation. The elevation in the records of discharge rate is due to stimulus artifacts.

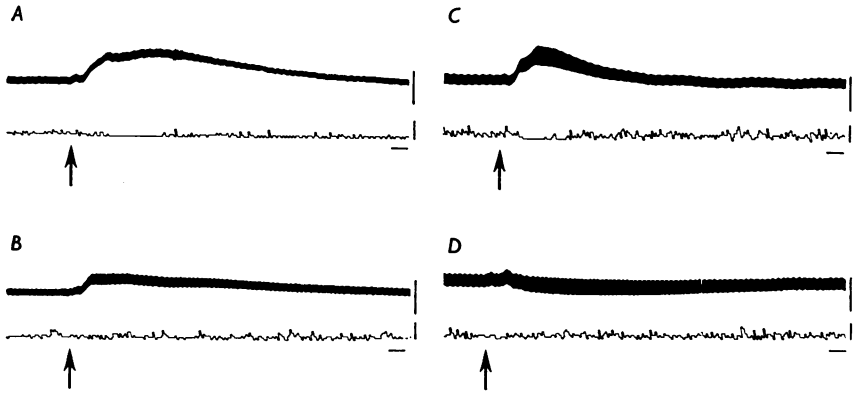

Fig. 6. Effect of alpha blocking agents on afferent response to intravenous (−)-adrenaline injection. Rabbit. *A* and *C* are control injections of 10 μg (−)-adrenaline. *B*. Test injection of 10 μg (—)-adrenaline 10 min after application of 1 mg ergotamine. *D*. Test injection of 10 μg (−)-adrenaline 40 min after application of 50 mg phenoxybenzamine.

(−)-adrenaline. It can be noted, however, that the pulse pressure was decreased in the latter test response. This might indicate that propranolol was effective in blocking the adrenergic influence on the contractile force of the heart.

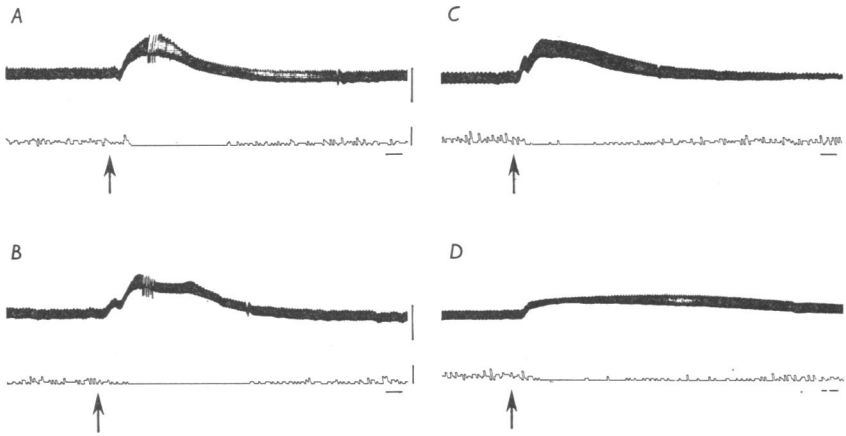

Fig. 7. Effect of beta blocking agents on the adrenaline response. Rabbit. *A* and *C* are control injections of 10 μg (−)-adrenaline. *B*. Test injection of 10 μg (−)-adrenaline after application of 15 mg of DCI. *D*. Test injection of 10 μg (−)-adrenaline after application of 5 mg of propranolol.

DISCUSSION

While there are numerous reports in the literature on the course and distribution of efferent fibres to the adrenal gland, there is a striking absence of information about afferent fibres. We have found but two brief mentions of adrenal afferents (Kiss, 1951 and Sato, 1952). Direct anatomical evidence of adrenal afferents has been found in this laboratory. Following suction aspiration of the adrenal medulla in the new-born pup, it is possible to find marked chromatolytic changes in scattered cells in the ipsilateral dorsal root ganglia (J. F. Cummings, personal communication). The distribution of ganglia having the greatest density of chromatolytic cells is quite similar to the distribution of preganglionic cells in the intermediolateral column showing greatest chromatolytic changes.

The records presented in this paper and elsewhere (A. Niijima & D. L. Winter, to be published) offer physiological confirmation that adrenal afferents are present. Thus far, three types of stimuli have proved effective in activating or modifying unitary activity. Mechanical distension of the adrenal capsule evokes a phasic, rapidly adapting train of spikes in some units. These units are ordinarily silent and do not respond to changes in blood pressure or catecholamine administration. They are presumably

mechanoreceptors located in the capsule or parenchyma of the gland. Two types of units have been found which are spontaneously active. One which appears to be sensitive to changes in blood pressure levels in the adrenal artery has been described elsewhere. This unit has baroreceptor-like properties and probably functions in that capacity as part of a local reflex concerned with the regional regulation of blood flow.

The function of the unit described in this report is more difficult to understand. The normally spontaneous firing rate is modified by catecholmine injections or other procedures which presumably change the catecholamine levels in the adrenal gland effluent. The mechanism of this action is certainly unclear. A distinct possibility is that it involves a receptor sensitive to either passive distension of arterial walls or to changes in tonicity of these walls. The lack of response following mechanically induced blood pressure changes would seem to disfavour the first possibility. The second possibility is similarly difficult to support in view of the lack of neuronal response to isoprenaline injection. This vasodilator had no effect on the discharge rate. A receptor sensitive to tension changes in the arterial wall would be expected to reflect the vasodilatation by a modification of its discharge rate.

Since these possibilities cannot totally be disproved, we have called the receptor chemosensitive. Additional experimentation will ultimately establish whether or not there is a true chemoreceptor in the adrenal gland.

An increasing number of reports have appeared which suggest that cells in the central nervous system are sensitive to adrenaline and noradrenaline. Depression of the patella reflex following intravenous injections of adrenaline was described by Schweitzer & Wright in 1937. This has been confirmed by several authors. McLennan (1961) has shown that this reflex depression following intravenous adrenaline and noradrenaline administration could be antagonized by an alpha blocking agent phenoxybenzamine, but not by a beta blocking agent dichloroisoprenaline (DCI). Noradrenaline administered by micro-electrophoretic techniques has been shown to depress the responses of some spinal cord interneurones to local applications of excitant amino acid (Engberg & Ryall, 1966). Motoneurone activation has been depressed by noradrenaline administered electropheretically (Weight & Salmoiraghi, 1967). Neurones in the caudate nucleus, also, are depressed by noradrenaline and dopamine. Bloom, Costa & Salmoiraghi (1965) and McLennan & York (1967) have found that approximately 60% of cells tested were depressed by dopamine. The latter authors have demonstrated that phenoxybenzamine is effective in blocking the dopamine depression of firing rate, while DCI is not.

The above studies, performed in the central nervous system proper, offer rather convincing evidence that catecholamines are capable of modifying

neuronal discharge rates. The sign of this action is predominantly depressive. We interpret the findings presented in this report to represent another example of this action, and suggest that the locus of the catecholamine effect is directly on the neurone which, in this case, is a sensory receptor. The specificity of this effect has not been established. However, it is clear that alpha blocking agents can eliminate the action of adrenaline and noradrenaline, while beta blocking agents cannot.

Whether these receptors respond directly or indirectly to catecholamines, their firing rates do seem to reflect catecholamine levels. The location of these receptors in the adrenal gland, the major secretory organ for catecholamine release, suggests the possibility of a local feed-back system. This possibility is supported by the anatomical data mentioned above which demonstrate a distribution of adrenal afferents corresponding, in general, to the distribution of adrenal efferents. The degree of participation of a neural feed-back system in the release of adrenal catecholamines is unknown. The existence of such a system, however, does offer new interpretations to studies of adrenal gland physiology.

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