J. Pharm. Pharmac., 1973, **25**, 537–543 *Received February* 8, 1973

The effect of corticosterone on extraneuronal amine uptake and effector response in rat salivary glands

OLLE ALMGREN AND JAN JONASON

Department of Pharmacology, University of Göteborg, Göteborg, Sweden

The effect of corticosterone on effector cell response to noradrenaline *in vivo* and on extraneuronal amine uptake *in vitro* has been investigated in rat submaxillary glands. When tissue slices were incubated with [^{3}H]noradrenaline the level of extraneuronally retained radioactive material was found to be markedly reduced at a concentration of 10 μg ml^{-1} of corticosterone after inhibition of neuronal uptake by protriptyline. Corticosterone in a dose of 10 mg kg^{-1} was found to markedly potentiate the secretory response to noradrenaline *in vivo*, when the neuronal uptake of noradrenaline was blocked by protriptyline (10 mg kg^{-1}, i.p.). Inhibition of neuronal uptake alone by protriptyline or of the extraneuronal uptake alone by corticosterone in the doses used here did not affect the dose-response curve for noradrenaline, at least not in its lower part. The data thus clearly show that the extraneuronal amine uptake of rat salivary glands is blocked by corticosterone and that this extraneuronal uptake might be regarded as a mechanism of importance for the inactivation of the adrenergic transmitter.

The extraneuronal amine uptake in sympathetically innervated organs, first observed in rat salivary glands (Andén, Carlsson & Waldeck, 1963) and later in several other tissues, although in less pronounced amounts (Almgren & Jonason, 1971a), probably in part corresponds to the Uptake$_2$ of Iversen (Iversen, 1965; Ehinger & Sporrong, 1968; Farnebo & Malmfors, 1969; Lightman & Iversen, 1969). The physiological significance of the extraneuronal amine uptake is as yet dubious but it may play an important role in the inactivation of the adrenergic transmitter in certain tissues (Iversen, Fisher & Axelrod, 1966; Almgren & Jonason 1971b, 1973; Kaumann, 1972; Hughes, 1972).

The observation by Kalsner (1969a, b) that hydrocortisone and some other adrenal cortical steroids potentiate responses to adrenaline and noradrenaline in rabbit aortic strips led Iversen & Salt (1970) and Salt (1972) to investigate the effect of some steroids on Uptake$_2$ of the rat heart. Corticosterone was found to be an effective inhibitor of this amine uptake. In view of this, the reports by Kaumann (1972) that hydrocortisone potentiated some cardiac effects of noradrenaline and isoprenaline in cats, and by Hughes (1972) that corticosterone induced a large increase of the noradrenaline overflow after field stimulation of the rabbit vas deferens, especially if the neuronal uptake was inhibited by cocaine, indicate that the extraneuronal amine uptake of several tissues may be of significant physiological importance. We have investigated this effect of corticosterone in the rat submaxillary gland, where its effect on both extraneuronal amine uptake and on effector response can be assessed.

METHODS

In vitro *studies.* Adult male Sprague-Dawley rats, about 250 g, were killed by a blow on the head and the submaxillary plus the sublingual glands were removed, weighed and immediately sliced (thickness about 0·5 mm). The slices (all those from one gland in a single flask) were preincubated in 5 ml of Krebs-Henseleit solution for 10 min at 37° in 5% carbon dioxide in oxygen (Rutledge & Jonason, 1967). In some experiments corticosterone was added to the incubation medium before preincubation. After the preincubation period, (±)-noradrenaline-7-^{3}H ($0{\cdot}5 \times 10^{-6}$ Ci) (^{3}H-NA) was added to give a final concentration of 10^{-7} M, and the tissues were incubated for 20 min. In one series of experiments, when ^{3}H-NA was used as a substrate, protriptyline (5 μg) was added to the medium before preincubation to prevent neuronal uptake of the amine. Control samples were prepared by addition of 2 ml 2N HCl to the flasks before preincubation. After 20 min incubation the slices were washed and further treated (Almgren & Jonason, 1971a). The total radioactivity of the slices was measured in a Tri-Carb Scintillation Counter. The values for retention of ^{3}H-NA or metabolic products were corrected for counting efficiency, and the specific activity of the substrate; the control values were subtracted from the experimental values. The *P*-values were calculated by *t*-tests after one-way analysis of variance (Davies, 1949).

Secretory responses. Male Sprague-Dawley rats, about 250 g, were anaesthetized with urethane (about 1 g kg^{-1}, i.p.). Under a dissecting microscope the duct of the submaxillary gland of one side was isolated and cannulated with a fine glass cannula (outside diameter 0·5 mm) according to Ohlin (1965). The femoral vein of one side was catheterized with a Portex polyethene catheter (PP 25, outside diameter 0·80 mm) by which intravenous injections of the agonist were given. The saliva appearing from the glass cannula in response to the injections of the agonist was collected in small plastic tubes which were weighed before and after the experiments. To obtain the peak response, the collection time was limited to 15 s from the moment saliva began to pour out from the tip of the cannula (a few seconds after the injection). Dose-response curves for noradrenaline (0·05–50 μg of the base) were obtained from four different groups of rats: untreated control rats, rats pretreated with corticosterone [(10 mg kg^{-1}, i.p.) (Ciba-Geigy, Basle) dissolved in ethanol–water giving a final ethanol concentration of 15% (v/v)] 30 min before the experiment, rats pretreated with protriptyline (10 mg kg^{-1}, i.p.) 25 min before, and rats pretreated with a combination of corticosterone and protriptyline as above. In some control experiments dose-response curves for noradrenaline were measured after treatment with a corresponding dose of ethanol (10 mg kg^{-1}, i.p. of a 15% solution). The solutions of the agonists were made so as to not exceed an injected volume of 0·5 ml at each injection. When noradrenaline was injected into untreated animals or those pretreated with corticosterone only, a higher dose was not given until the secretory response to the preceding dose had ceased. The response to the noradrenaline injections had a long duration after pretreatment with protriptyline alone or with corticosterone and thus it was considered more accurate in these instances to give cumulative doses of noradrenaline without an interval between injections longer than was needed to collect the saliva for 15 s. The actual doses of noradrenaline given to these rats were calculated by using the difference between intended dose level and the sum of the preceding doses given. The possible error so induced seems small

judging from control experiments and should act to diminish a potentiation by corticosterone and protriptyline rather than to increase it.

It was not possible to obtain full dose-response curves in these experiments, since high doses of noradrenaline (>50 μg in the untreated animals and >5–10 μg in the pretreated rats) caused a high mortality rate and often gave much lower secretory responses than the preceding dose. This effect was interpreted as the result of cardiovascular disturbances arising from the high doses of noradrenaline.

The curves in Fig. 1 are drawn according to the mean values of all experiments. The statistical comparison was made by analysis of variance on the calculated dose levels for each experiment corresponding to a secretory response of 10 mg saliva g^{-1} salivary gland (D_{10}) and 50 mg saliva g^{-1} salivary gland (D_{50}), respectively.

After the experiments the rats were killed and the salivary glands were weighed. The amount of saliva in each sample was related to the weight of the salivary gland.

RESULTS

The effect of corticosterone upon the retention of the radioactivity in rat salivary gland slices after incubation with ^{3}H-NA is presented in Table 1. In the controls, $262{\cdot}4 \times 10^{-12}$ mol of ^{3}H-NA (or metabolic products) were retained. Corticosterone

Table 1. *Effect of corticosterone in different concentrations upon the retention of radioactivity in rat salivary glands after incubation with [^{3}H]noradrenaline.* Rat salivary gland slices were incubated for 20 min with 10^{-7} M[^{3}H]noradrenaline in Krebs-Henseleit solution in the presence of different concentrations of corticosterone. In one series of experiments protriptyline was added to the incubation medium before the incubation. The values represent the amount of radioactivity retained by the slices after a 10 min postincubation in a substrate-free medium and are expressed as mol $\times$ 10^{-12} g^{-1} tissue, n represents the number of experiments.

Corticosterone (μg ml^{-1})		Without protriptyline	With protriptyline 1 μg ml^{-1}
0	Mean	262·4	65·96
	s.e.	27·30	8·17
	n	4	4
0·1	Mean	303·2	76·26
	s.e.	16·45	7·56
	n	4	4
1	Mean	294·5	56·77
	s.e.	17·24	2·04
	n	4	4
10	Mean	304·2	35·76**
	s.e.	17·56	2·52
	n	4	4
50	Mean	263·4	24·60***
	s.e.	19·05	1·94
	n	4	4

** Significantly different from controls at $P < 0{\cdot}005$.

*** At $P < 0{\cdot}001$.

in the concentrations used did not significantly affect this retention in the experiments without protriptyline. After inhibition of neuronal amine uptake by protriptyline (1 μg ml^{-1}) the retention of radioactive material in the controls was reduced to 25% of normal. Corticosterone, 0·1 and 1 μg ml^{-1} did not affect this retention. Ten μg ml^{-1} of corticosterone significantly reduced the amount of radioactive material retained to 57% (P <0·005). A further reduction to 37% was obtained at the highest corticosterone concentration used (50 μg ml^{-1}) (P <0·001).

The secretion of saliva after various doses of noradrenaline is represented in dose-response curves in Fig. 1. Doses higher than 50 μg alone caused the secretory

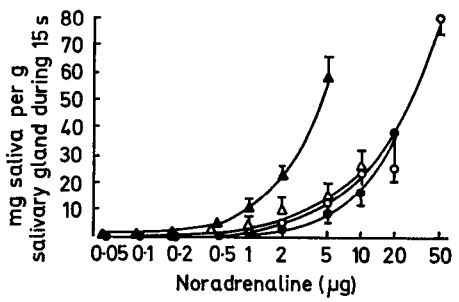

FIG. 1. Secretory response in rat submaxillary gland to noradrenaline injections. The excretory duct of rat submaxillary glands was cannulated and the saliva was collected during 15 s after the i.v. injection of different doses of noradrenaline. The animals were anaesthetized with urethane and some rats had no pretreatment (O), some were pretreated with 10 mg kg^{-1} of corticosterone i.p. 30 min before the experiment (●), and some with 10 mg kg^{-1} of protriptyline i.p. 25 min before the experiment (△) or with a combination of both (▲). The results are expressed as mg saliva g^{-1} submaxillary gland and the mean values are indicated. The vertical lines represent s.e. The number of experiments is given in Table 2.

response of the normal rat to decline, and this effect was seen after 20 μg when the rats were pretreated with corticosterone, after 10 μg when the rats were pretreated with protriptyline and after 5 μg when the rats were pretreated with both drugs.

The dose-response curve obtained from rats pretreated with 10 mg kg^{-1} corticosterone 30 min before the experiments did not differ from the control curve at low dose levels, *i.e.* less than 20 μg noradrenaline. At a dose of noradrenaline of 20 μg, the response was markedly increased in some corticosterone pretreated rats compared to controls. After still higher doses of noradrenaline the response declined. Pretreatment with protriptyline, 10 mg kg^{-1} 25 min before the experiment, did not significantly change the position of the dose-response curve in relation to the control. The curve from animals pretreated with both corticosterone and protriptyline was shifted to the left of the control curve over the entire dose range. D_{10} and D_{50} for the different dose-response curves are given in Table 2. D_{10} in the protriptyline-treated animals did not differ significantly from D_{10} in the controls. D_{50} was not obtained after protriptyline pretreatment. After pretreatment with corticosterone, D_{50} was significantly lower than in the controls (P <0·05), although in only two dose-response curves obtained after corticosterone was a response of 50 ng g^{-1} saliva or more reached before the response again declined. After pretreatment with the combination of corticosterone and protriptyline both D_{10} and D_{50} were much lower than in the controls (P <0·001).

Table 2. *Mean values of D_{10} and D_{50}, analysis of variance and significance of differences between the groups calculated from the data given in Fig. 1.* The values are calculated from individual dose-response curves from the experiments shown in Fig. 1. D_{10} and D_{50} are the dose levels of noradrenaline required to give a secretory response of 10 and 50 mg saliva g^{-1} salivary gland, respectively. The figures in brackets are the number of experiments. The difference in number of experiments from each group between D_{10} and D_{50} is due to the fact that some experiments were not pursued throughout the whole dose range and did not reach this effect level. $Ø_1$ and $Ø_2$ represent the degrees of freedom between groups and within groups, respectively.

	Noradrenaline dose (μg)				Analysis of variance			
Effect level	Control	Protriptyline (10 mg kg^{-1})	Cortico-sterone 10 mg kg^{-1}	Protriptyline (10 mg kg^{-1}) cortico-sterone (10 mg kg^{-1})	F	Variance within groups	$Ø_1$	$Ø_2$
D_{10}	6·2 (29)	6·3 (5)	7·2 (8)	1·1*** (9)	5·39	14·64	3	47
D_{50}	22·1 (6)	—	17·4* (2)	5·7*** (8)	65·22	6·01	2	13

*** Significantly different from control at $P < 0{\cdot}001$.

* $P < 0{\cdot}05$.

In the two control experiments, where ethanol (10 ml kg^{-1}, i.p. of a 15% solution) was administered, no shift of the dose-response curve to noradrenaline was observed compared with the untreated rats.

DISCUSSION

The concentration of protriptyline used in the *in vitro* work (1 μg ml^{-1}) as well as the dose used *in vivo* (10 mg kg^{-1}) is known to effectively prevent neuronal uptake of noradrenaline in rat salivary glands (Almgren & Jonason, 1971a, b). The retained radioactivity of salivary gland slices after incubation with 10^{-7} M ^{3}H-NA represents mainly intraneuronally located noradrenaline, if the neuronal amine uptake mechanism is intact, but mainly extraneuronally located normetanephrine, if the neuronal uptake mechanism is blocked (Almgren & Jonason, 1971b). The existence of a second uptake process located at the effector cells is nowadays well established (for ref. see introduction), but the functional importance of this is not yet fully understood. Normetanephrine is known to block this mechanism in different tissues (Iversen, 1965; Draskoćzy & Trendelenburg, 1970; Gillespie, Hamilton & Hosie, 1970). Recently, it was shown that normetanephrine reduced extraneuronal uptake of noradrenaline also in rat salivary glands and this reduction was associated with a potentiation of the secretory response to noradrenaline, indicating a functional importance of this mechanism (Almgren & Jonason, submitted for publication).

A potentiating effect by adrenal cortical steroids on physiological responses to sympathomimetic amines has been described (see *e.g.* Zweifach, Shorr & Black, 1953;

Reis, 1960; Kalsner, 1969a, b; Kaumann, 1972). Recently an inhibitory effect of these steroids on the $Uptake_2$ of the rat heart was reported (Iversen & Salt, 1970; Salt, 1972). In the present study a marked potentiation of the secretory response in the rat submaxillary gland to noradrenaline was observed after treatment with corticosterone, at least if the neuronal uptake was also inhibited by protriptyline. If evenly distributed, the dose of corticosterone used (10 mg kg^{-1},) agrees fairly well with the concentration giving a marked inhibition of extraneuronal amine uptake *in vitro* (10 μg ml^{-1}). Thus, it seems likely that these two effects are causally related. Interestingly, in preliminary studies no inhibitory effect on extraneuronal uptake has been found with hydrocortisone, which is not secreted in rats; it is corticosterone which is the main naturally occurring glucocorticoid in this animal (Salt, 1972). If this effect on extraneuronal uptake by the corticoids is related to their endogenous occurrence in rats, this might indicate a physiological significance of this effect of the adrenal corticoid hormones.

Kalsner (1969b) has shown that inhibition of catechol-*O*-methyl transferase (COMT) induced a potentiation of the contractile response of rabbit aortic strips to adrenaline of the same magnitude as the potentiation seen after hydrocortisone. On this basis he suggested that inhibition of COMT could be a mechanism of action for this effect of the adrenal corticoids. Impaired inactivation of the amines by reduced uptake into the tissues seems now to be a more probable explanation, as was also suggested by Kalsner (1969b), since hydrocortisone also potentiated the response to phenylephrine, which is not a substrate for COMT. Furthermore, the marked increase of the noradrenaline overflow in the rabbit vas deferens or the rabbit portal vein following field stimulation after corticosterone (Hughes, 1972) strongly supports the hypothesis of an impaired uptake mechanism by the steroids. Also the finding that corticosterone potentiated the noradrenaline overflow in these preparations much more effectively if the neuronal uptake was also blocked by cocaine, is in good agreement with the reported balance between neuronal and extraneuronal uptake in rat salivary glands (Almgren & Jonason, 1971b).

The present study has demonstrated that the main adrenal cortical steroid in the rat, corticosterone, inhibits the extraneuronal uptake of noradrenaline in rat salivary glands. This effect appears to be associated with a potentiation of the secretory response to noradrenaline. The data support the view that the extraneuronal uptake mechanism is of functional importance in some tissues. This effect may be of importance for the physiological role of the adrenal corticoids in stress reactions. In view of the frequent clinical use of the steroids, such an effect should also be considered as a possible mechanism for eliciting drug interactions.

Acknowledgements

The research reported in this study has been sponsored by the Swedish State Medical Research Council (No. 04X-2862), the Medical Faculty, University of Göteborg, and "Wilhelm och Martina Lundgrens Vetenskapsfond". The expert technical assistance of Mrs. Gunilla Eriksson, Mrs. Gunilla Jonason and Miss Birgit Samuelsson is gratefully acknowledged. The generous gift of protriptyline from Merck, Sharp & Dohme Int. and of corticosterone from Ciba-Geigy, Basle, is gratefully acknowledged.

REFERENCES

ALMGREN, O. & JONASON, J. (1971a). *Acta physiol. scand.*, **82**, 282–288.

ALMGREN, O. & JONASON, J. (1971b). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **270**, 289–309.

ANDÉN, N.-E., CARLSSON, A. & WALDECK, B. (1963). *Life Sci.*, **2**, 889–894.

DAVIES, O. L. (1949). *Statistical Methods in Research and Production* (2nd edn.), p. 96, London: Oliver & Boyd.

DRASKOĆZY, P. R. & TRENDELENBURG, U. (1970). *J. Pharmac. exp Ther.*, **174**, 290–306.

EHINGER, B. & SPORRONG, B. (1968). *Experientia*, **24**, 265–266.

FARNEBO, L.-O. & MALMFORS, T. (1969). *Eur. J. Pharmac.*, **5**, 313–320.

GILLESPIE, J. S., HAMILTON, D. N. H. & HOSIE, R. J. A. (1970). *J. Physiol. (Lond.)*, **206**, 563–590.

HUGHES, J. (1972). *Br. J. Pharmac.*, **44**, 472–491.

IVERSEN, L. L. (1965). *Br. J. Pharmac. Chemother.*, **25**, 18–33.

IVERSEN, L. L., FISHER, J. E. & AXELROD, J. (1966). *J. Pharmac. exp. Ther.*, **154**, 56–63.

IVERSEN, L. L. & SALT, P. J. (1970). *Br. J. Pharmac.*, **40**, 528–530.

KALSNER, S. (1969a). *Ibid.*, **36**, 582–593.

KALSNER, S. (1969b). *Circ. Res.*, **24**, 383–395.

KAUMANN, A. J. (1972). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **273**, 134–153.

LIGHTMAN, S. L. & IVERSEN, L. L. (1969). *Br. J. Pharmac.*, **37**, 638–649.

OHLIN, P. (1965). *Acta Univ. Lund II*, **23**, 1–12.

REIS, D. J. (1960). *J. clin. Endocr. Metab.*, **20**, 446.

RUTLEDGE, C. O. & JONASON, J. (1967). *J. Pharmac. exp. Ther.*, **157**, 493–502.

SALT, P. J. (1972). *Eur. J. Pharmac.*, **20**, 329–340.

ZWEIFACH, B. W., SHORR, E. & BLACK, M. M. (1953). *Ann. N.Y. Acad. Sci.*, **56**, 626.