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MECHANISMS OF INACTIVATION OF NORADRENALINE IN THE IRIS SPHINCTER, TRACHEAL MUSCLE AND FACIAL ARTERY OF CATTLE: IMPLICATIONS FOR β-ADRENOCEPTOR-MEDIATED RESPONSES

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1 The role of neuronal and extraneuronal pathways of amine inactivation in regulating the inhibitory actions of noradrenaline was investigated in three bovine smooth muscle preparations in which the primary adrenoceptor is of the β-type.

2 The extraneuronal uptake inhibitor, 17β-oestradiol, sensitized the inhibitory responses to noradrenaline in the facial artery, the iris sphincter and in tracheal muscle preparations, indicating a major role for non-neuronal processes in agonist-inactivation in all three preparations. Cocaine also increased responses to noradrenaline, pointing to a role for neuronal uptake either as a terminating mechanism or as a process limiting access of exogenous agonist molecules to their site of action.

3 Cocaine did not enhance significantly responses to isoprenaline, a potent β-adrenoceptor agonist which is not taken up neuronally. Further, relaxations to metaraminol, a sympathomimetic amine which is taken up extraneuronally, but much less so than noradrenaline, were also less enhanced by 17β-oestradiol in the three preparations tested. These findings support the specificity of action of cocaine and 17β-oestradiol as neuronal and extraneuronal uptake inhibitors in the present experiments.

4 Studies of the uptake of [^{3}H]-noradrenaline revealed that 17β-oestradiol reduced the uptake of amine in the presence of cocaine, confirming a cocaine-resistant site of action for the steroid in all three preparations.

5 It is concluded that extraneuronal uptake sites are located sufficiently close to the β-adrenoceptors to modulate the concentration and duration of action of noradrenaline at these sites of action. It is proposed that in smooth muscles which contain a preponderance of β-receptors, extraneuronal metabolism is a key event in terminating the inhibitory effects produced.

Introduction

The uptake and metabolism of noradrenaline at extraneuronal sites is important in terminating its action in vascular smooth muscle (Kalsner & Nickerson, 1969; Gillespie, Hamilton & Hosie, 1970; Burnstock, McCulloch, Story & Wright, 1972; Bevan & Su, 1973; Kalsner, 1976; 1977). However, the exploration of the relative roles of neuronal and extraneuronal processes of catecholamine inactivation in other smooth muscles is limited and much of the early data describing the avidity of adrenergic nerve terminals for noradrenaline and the quantification of metabolites is of little value in assessing the dynamics and concentrations of agonist at the postsynaptic receptor sites during the course of the response (Kalsner, 1976; 1977). The function of individual inactivation processes in ending agonist action needs to be assessed by determining directly the effects of inhibiting them on response parameters in specific organ systems.

In the present experiments, three distinct smooth muscle preparations, all of which relax in response to noradrenaline-mediated β-receptor activation, were studied *in vitro* as a follow-up of previous work on the extraneuronal inactivation of noradrenaline in the coronary arteries of cattle (Kalsner, 1974a). The finding that extraneuronal removal of agonist is a significant terminating mechanism in the iris sphincter, tracheal muscle and facial artery, as well as in the coronaries, suggests that a generalization can now be made for β-receptor-mediated responses in smooth muscle and routes of noradrenaline inactivation.

Methods

Tissue preparation

Tracheae, transverse facial arteries and eyes were removed rapidly, after slaughter, from beef cattle of either sex, immersed in oxygenated Krebs solution and transported to the laboratory (total time approximately 20 min). For the tracheal preparation, a length was cut into rings and the layer of tissue on the inner surface of each ring was dissected out, cut in half and trimmed to two 4.0 cm strips. The strips were then suspended under 2 g tension in 15 ml muscle chambers at 37°C containing Krebs-Henseleit solution continuously bubbled with 95% O_2 and 5% CO_2. The iris sphincter (circular) muscles were dissected out and tied at each end as described by Kern (1970) and mounted under 1 g tension in 15 ml muscle chambers as described above. The facial arteries, after removal, were cleaned of visible fat and adherent tissue, and cut into spiral strips of about 25 × 2.5 mm. Both left- and right-hand spirals were cut with no detectable difference in any parameter of response. The strips of artery were mounted in muscle chambers in the same way as the trachea. For all preparations, isotonic contractions and relaxations were recorded by means of frontal writing levers on a slowly moving kymograph drum (about 1.8 mm/min), with a lever magnification of 6.8 fold.

Drugs

The following drugs were used: acetylcholine chloride (Calbiochem); cocaine hydrochloride (BDH); (±)-isoprenaline (Winthrop); metaraminol (Merck, Sharp & Dohme); (−)-noradrenaline bitartrate (Calbiochem); (±)-[7-^{3}H]-noradrenaline bitartrate (New England Nuclear Corp.); 17β-oestradiol (Calbiochem); phenoxybenzamine hydrochloride (Smith Kline & French); phentolamine hydrochloride (Ciba-Geigy); (−)-(isopropylamine)-3-(α-naphthoxy)-2-propanol hydrochloride (propranolol hydrochloride, Aldrich); sotalol (Mead Johnson). The modified Krebs-Henseleit solution had the following composition (mM): NaCl 115.3; KCl 4.6; $CaCl_2$ 1.8; $MgSO_4$ 1.1; $NaHCO_3$ 22.1 and glucose 7.8, to which disodium edetate (EDTA, 0.01 g/l) was added.

All drug concentrations are expressed in terms of molarity. Cocaine hydrochloride was dissolved to the appropriate concentration in distilled demineralized water, (−)-noradrenaline bitartrate was diluted to the desired concentration in 0.01 N HCl and 17β-oestradiol was prepared in ethanol to give a stock concentration of 37 mM. The volume of ethanol used (0.015 ml) had no effect on the basal tone of the strips. All drug concentrations refer to final concentrations in the muscle chambers. Preparations were used to obtain only one dose-response curve to a given agonist and then discarded.

[^{3}H]-noradrenaline uptake protocol

(±)-[7-^{3}H]-noradrenaline bitartrate (8.7 or 11.4 mCi/mol) was used to study [^{3}H]-noradrenaline uptake by tracheal, iris sphincter muscles and facial arteries. The noradrenaline was diluted to a stock concentration of 1 μM in ascorbic acid (50 μg/ml) and stored frozen in 4 ml aliquots under nitrogen. Aliquots were thawed only once, immediately before use. For uptake studies, the iris sphincter and tracheal tissues were prepared as described above and the facial arteries were cut longitudinally into four strips of approximately equal size. Tissues were then preincubated individually at 37°C for 60 min in vials containing 10 ml Krebs solution.

Each preparation was then divided into three groups; two groups were preincubated for 20 min with cocaine (29 μM) and one group served as control. One of the cocaine-treated groups was also pre-incubated with 17β-oestradiol (37 μM) for 20 min. All tissues were then incubated with [^{3}H]-noradrenaline (6×10^{-7} M, i.e. 3×10^{-8} M (±)-[^{3}H]-NA diluted 20 times with non-radioactive (±)-NA) for a period of 20 min, then rinsed, gently blotted and weighed. The tracheal muscle and facial artery tissue were chopped and placed in scintillation vials containing 2 ml of solubilizer (Protosol, New England Nuclear Corp) and 0.2 ml of distilled water to promote dissolution of the tissue. The vials were then kept overnight in a water bath at 50°C to speed solubilization.

Individual iris sphincter muscles were chopped and ground in a mortar with 1.5 g washed sea sand and 3 ml of *n*-butanol-0.1% HCl. The homogenate was transferred to a centrifuge tube and the mortar and pestle were washed twice with 2 ml aliquots of *n*-butanol-0.1% HCl. The washings were added to the homogenate and centrifuged at high speed (5100 *g*) in a table-top IEC clinical centrifuge for 15 min. The supernatant was decanted and saved and the pellet resuspended in 3 ml *n*-butanol-0.1% HCl and again centrifuged. The supernatants were combined, and 1.0 ml aliquots removed and placed in scintillation vials.

For all tissues, 15 ml of scintilation fluid (4 g 2.5-diphenyloxazole (PPO) and 50 mg (1,4-bis[2(5-phenyl-oxazolyl)]benzene (POPOP) per litre of toluene was added to each vial and the samples were counted to a 1% error in a Beckman LS-150 system with automatic external standardization, to determine efficiency. Uptake of radioactivity was expressed as disintegrations per min (d/min) and per g of tissue. The concentration of [^{3}H]-noradrenaline in the bathing medium was confirmed for each experiment by counting the radioactivity in 0.1 ml aliquots.

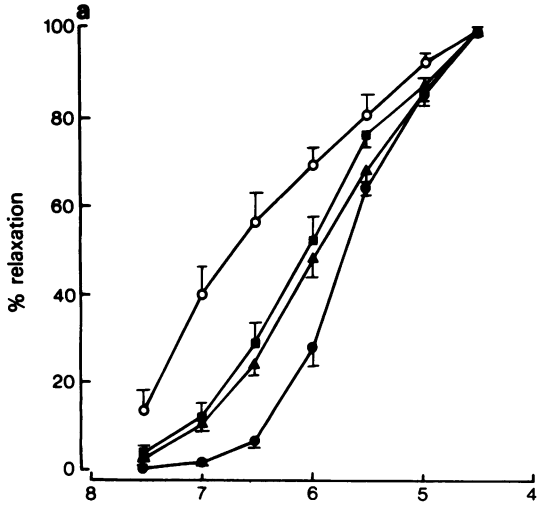
Data analysis

Mean values ± s.e. mean of all data are shown. Differences with P values of 0.05 or less were considered significant. Changes in response at the mean effective concentration (ED_{50}) are expressed as the ratio of geometric mean values (Fleming, Westfall, De La Lande & Jellett, 1972). Each ED_{50} was converted to its log and the mean for each group was recorded. The antilog of each mean log is presented as the geometric mean. Due to the lack of parallelism of certain of the dose-response curves, comparisons at other response levels would yield somewhat different values (Kalsner, 1974b). The contribution of neuronal and extraneuronal uptake to termination of action, as determined by the sensitization achieved, was calculated as described previously (Kalsner & Nickerson, 1968).

Results

Responses of circular iris muscle

The circular muscle of the bovine iris develops spontaneous tone with time, after mounting in muscle chambers. By 60 min, the plateau level of tone was usually achieved; it was a mean of 90.7 ± 3.6 mm in the 47 preparations tested. The adrenoceptors of the iris sphincter which are predominantly of the β-type inhibit tone (Schaeppi & Koella, 1963). This was confirmed in the present experiments by the finding that sotalol (37 μM) but not phentolamine (3.6 to 36 μM) or phenoxybenzamine (0.3 to 33 μM) antagonized the responses to noradrenaline. The concentration-response curve was shifted approximately 20 fold to the right by the β-receptor antagonist.



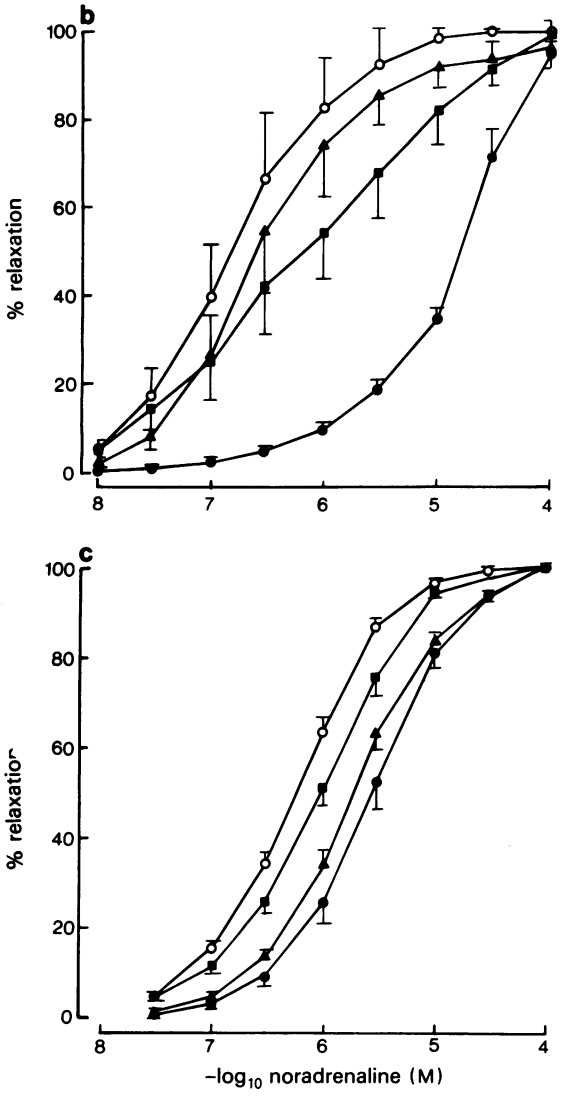


Figure 1 Effects of inhibitors of neuronal and extraneuronal uptake on cumulative concentration-response curves to noradrenaline in bovine iris sphincter (a), facial artery (b) and tracheal muscle (c). Control (●), cocaine pretreated (▲), 17β-oestradiol (37 μM) pretreated (■) and cocaine plus 17β-oestradiol pretreated (○). The concentration of cocaine was 29 μM except in facial arteries where it was 2.9 μM. Number of values in each group for iris sphincter, facial artery and trachea are 14, 15, 14, 4; 13, 6, 11, 5 and 13, 13, 16, 14, respectively. Vertical lines show s.e. means.

Progressively increasing concentrations of noradrenaline yielded relaxations of increasing magnitude in untreated preparations, as shown in Figure 1a.

The maximally effective concentration of the catecholamine (59 μM) produced a mean inhibition of 85.0 ± 4.1% of the total tone before the start of the agonist concentration-response curve. Washout of the noradrenaline from the muscle chambers led to the restoration of initial tone, requiring about 60 min for completion.

Blockade of noradrenaline inactivation

After equilibration in the muscle chambers, the preparations were exposed either to the neuronal uptake inhibitor cocaine (10 μg/ml; 29 μM) or to the blocker of extraneuronal uptake, 17β-oestradiol (10 μg/ml; 37 μM), or to the combination of inhibitors. Twenty min later, without washout of the muscle chambers, the concentration-response curves to noradrenaline were obtained. Treatment with cocaine shifted the concentration-response curve significantly to the left, indicating sensitization, as shown in Figure 1, but the inhibitor of extraneuronal uptake had an even greater effect. The ratio of geometric mean ED_{50} values (control/treated) was 1.5 and 2.1 after cocaine and 17β-oestradiol respectively (Table 1). Combined treatment with cocaine and 17β-oestradiol shifted the concentration-response curve further to the left than did either inhibitor alone, as can be seen both in Table 1 and Figure 1a.

Responses to and inactivation of noradrenaline in facial artery preparations

Facial artery strips rapidly gained tone and by 60 to 120 min after mounting, a plateau level was usually apparent; it was a mean of 72.5 ± 3.3 mm in the 35 preparations examined. The predominant adrenoceptor in this preparation is β, as the responses to noradrenaline, over a wide concentration range, are inhibitory, and blocked by propranolol (1.2 μM). After blockade of β-receptors, noradrenaline elicited small contractile responses which were antagonized by phentolamine (1.0 μM). In preliminary tests, cocaine (29 μM) caused some reduction in the tone of facial artery strips and for that reason the concentration routinely employed for blockade of neuronal uptake was 2.9 μM. Treatment of fresh strips with 17β-oestradiol or cocaine as was done with the iris muscle led to a highly significant shift to the left of the concentration-response curves as shown in Figure 1b and Table 1, and the combined inhibition of both neuronal and extraneuronal pathways had a somewhat greater sensitizing effect, probably obscured statistically by the size of the standard errors.

Responses to and inactivation of noradrenaline in tracheal muscle

Similar experiments to those on the iris were also carried out on bovine tracheal smooth muscle preparations. This tissue did not reliably develop a spontaneous tone and therefore, for the study of β-receptor-mediated responses, tone was routinely induced with acetylcholine (0.6 to 17 μM, usually 17 μM). The cholinomimetic drug was maintained in the muscle chamber throughout the experiment. The mean tone induced by acetylcholine, which was also the total

Table 1 Effects of neuronal and extraneuronal uptake inhibitors on the concentration-response curves to noradrenaline in the iris sphincter, facial artery and tracheal preparations of cattle

Preparation	*Treatment*	*No. of values*	*Geometric mean* ED_{50} ($\times 10^{-7}$ M)†	*Ratio of* ED_{50}*s*	P *value*	
				*	*	**
Iris sphincter m.	Control	14	7.9 ± 0.7			
	Cocaine	15	5.2 ± 0.7	1.5	<0.05	<0.001
	17β-Oestradiol	14	3.7 ± 0.8	2.1	<0.01	<0.01
	17β-Oestradiol + cocaine	4	1.0 ± 0.9	7.9	<0.001	
Facial artery	Control	13	88.7 ± 0.01			
	Cocaine	6	2.0 ± 0.01	44.4	<0.001	NS
	17β-Oestradiol	11	4.5 ± 0.01	19.7	<0.001	NS
	17β-Oestradiol + cocaine	5	1.2 ± 0.01	73.9	<0.001	
Trachea m.	Control	13	14.5 ± 0.8			
	Cocaine	13	6.6 ± 0.7	2.2	<0.01	<0.001
	17β-Oestradiol	16	4.5 ± 0.7	3.2	<0.001	<0.01
	17β-Oestradiol + cocaine	14	3.3 ± 0.7	4.4	<0.001	

* Comparison made with untreated control group. ** comparison made with the group treated with 17β-oestradiol + cocaine. † mean of concentrations producing 50% of maximal response to noradrenaline in each muscle strip.

tone, was 32.7 ± 2.0 mm in 56 preparations. The concentration-response curve to noradrenaline (59 nM to 59 μM) obtained in 13 control strips is shown in Figure 1c. The tracheal strips were relaxed by a mean of 85.0 ± 4.1% of their pre-existing tone in the presence of a maximally effective concentration of noradrenaline (59 μM). Cocaine (29 μM) shifted the concentration-response curve significantly to the left, but again, inhibition of extraneuronal uptake potentiated responses to noradrenaline to an even greater extent. The ratio of geometric mean ED_{50}s was 2.2 and 3.2, respectively, with the combination of the two inhibitors producing an effect significantly greater than that of cocaine alone (Figure 1c and Table 1).

Specificity of the enhancing agents

To confirm that the ability of cocaine to sensitize responses to noradrenaline is directly related to blockade of the neuronal uptake of the amine, experiments were done with isoprenaline, a potent β-adrenoceptor agonist with negligible affinity for the amine transport sites of adrenergic nerves (Iversen, 1965). Cocaine did not significantly alter the geometric mean ED_{50}s of concentration-response curves to isoprenaline, in any of the three preparations tested (Table 2).

Similarly, to establish whether the sensitization of responses induced by 17β-oestradiol is non-specific and thus common to all adrenoceptor agonists or, instead, related to the known relative affinity of amines for the extraneuronal transport sites, experiments were done with metaraminol (Table 2). This sympathomimetic amine is taken up extraneuronally, to only a negligible extent in heart (Iversen, 1965), although some accumulation appears to occur in vascular tissue (Kalsner, 1975).

The geometric mean ED_{50} of the iris sphincter for metaraminol was not altered by the steroid, although in the facial artery and trachea relatively slight but significant shifts of the ED_{50} ratios were observed. These findings probably indicate some capacity of the extraneuronal removal process to accept metaraminol, but even if a component of non-specific sensitization is involved here it appears to be of insufficient magnitude to alter substantially the findings with noradrenaline.

Uptake of [^{3}H]-noradrenaline into tissues

Direct evidence for the extraneuronal uptake of noradrenaline and its inhibition by 17β-oestradiol in bovine tracheal, facial artery and iris preparations was obtained in experiments with [^{3}H]-noradrenaline. A concentration of 6×10^{-7} M was chosen since it induces a response midway on the concentration-response curve in all three preparations. The comparison of untreated preparations with those exposed to cocaine revealed the anticipated inhibitory effect on uptake; presumably by blockade of entry into neuronal compartments (Figure 2). Additional treatment with the steroid significantly diminished uptake in the presence of cocaine, in all three preparations tested, confirmining an extraneuronal site of action for the steroid (Kalsner, 1969; Kalsner, Frew & Smith, 1975).

Table 2 Effects of neuronal and extraneuronal uptake inhibitors on the concentration-response curves to isoprenaline and metaraminol in the iris sphincter, facial artery and tracheal preparations of cattle

Preparation	*Agonist*	*Treatment*	*No. of values*	*Geometric mean* ED_{50} ($\times 10^{-7}$ M)†	*Ratio of* ED_{50}s	P *values*
Iris sphincter m.	Isoprenaline	Control	8	0.025 ± 0.006	*	*
		Cocaine	8	0.017 ± 0.006	1.5	<0.4
	Metaraminol	Control	6	6.1 ± 0.9		
		17β-Oestradiol	9	6.1 ± 0.8	1.0	NS
Facial artery	Isoprenaline	Control	4	0.032 ± 0.008		
		Cocaine	4	0.017 ± 0.006	1.9	<0.4
	Metaraminol	Control	15	20.9 ± 0.7		
		17β-Oestradiol	14	7.2 ± 0.7	2.9	<0.01
Trachea m.	Isoprenaline	Control	7	0.412 ± 0.06		
		Cocaine	7	0.492 ± 0.06	0.8	NS
	Metaraminol	Control	13	48.4 ± 0.7		
		17β-Oestradiol	12	26.3 ± 0.7	1.8	<0.01

* Comparison made with untreated control group. † Mean of concentrations producing 50% of maximal response to isoprenaline and metaraminol in each muscle strip.

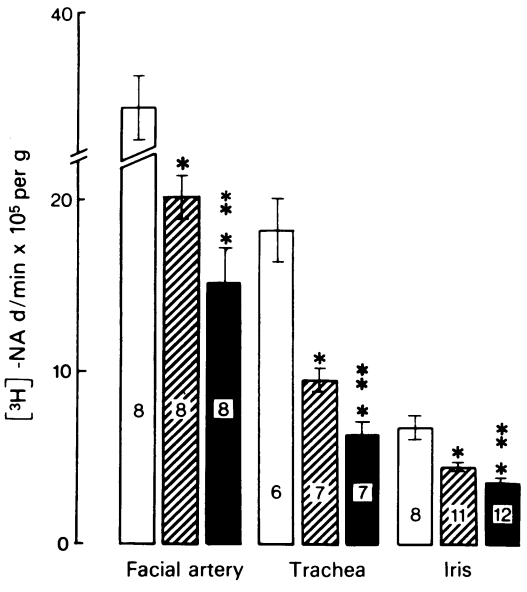


Figure 2 Effects of inhibitors of neuronal and extraneuronal uptake on the uptake of [^{3}H]-noradrenaline 6×10^{-7} M in iris sphincter, facial artery and tracheal muscle. Means are presented; vertical lines show s.e. means. Open columns represent controls, hatched columns cocaine and solid columns cocaine plus 17β-oestradiol (37 μM)-treated preparations. The concentration of cocaine was 29 μM except in facial arteries where it was 2.9 μM. *Values significantly different from controls; **significant difference between cocaine plus 17β-oestradiol-treated groups and those treated only with cocaine.

Discussion

There are limitations in the present approach to an analysis of terminating mechanisms. Termination of action is a physiological event which is formally assessed by the direct measurement of the time taken for a preparation to recover from a response mediated by receptor activation. If desensitization is ruled out as a contributing factor, as it can be in the design of the present experiments where sensitization is clearly observed, and if agonist dissociation from receptors is not a limiting factor, as it does not appear to be with the adrenoceptors (Furchgott, 1955; Kalsner, 1976), then termination of action is determined by the time taken for the active agonist concentration in the region of the receptors to decline towards zero.

The present experiments involved the study of β-receptor-transmitted inhibitory responses but direct analysis of the decay of the response was not feasible. The process of recovery, after washout of agonist, back towards the level of tone existing prior to the agonist-induced relaxation is sufficiently unpredictable, due to the unknown variables which determine spontaneous tone, to make the study of the magnitude of inhibitory responses and their sensitization a more precise, although only circumstantial, measure of agonist inactivation pathways and their interruption. However, the caveats raised elsewhere (Kalsner, 1976; 1977) concerning the difficulties in distinguishing between effects on access pathways from those on terminating mechanisms and the complexities introduced by the possible presence of alternate and multiple pathways of inactivation apply here. For example, blockade by cocaine of an active neuronal uptake process, which acts as a screen filtering out agonist molecules en route to receptors, may substantially add to the component of sensitization which is attributed to neuronal uptake functioning as a terminating mechanism. Thus, the role of neuronal uptake is probably overestimated in the experiments recorded here. Such is unlikely to be equally the case with extraneuronal uptake, a process which appears to be less distant from the adrenoceptors on the effector cells.

The cholinergically innervated iris sphincter muscle of diverse species also contains adrenergic neurones, as evidenced by fluorescence histochemistry (Laties & Jacobowitz, 1966) and the presence of axons containing dense core granules and their deterioration following superior cervical ganglionectomy (Ochi, Konishi, Yoshikawa & Sano, 1968; Nishida & Sears, 1969; Geltzer, 1969). The bovine iris sphincter responds to electrical stimulation, in the presence of atropine, with relaxation (Gruenhagen & Samkowy, 1875) and human and cat iris sphincter contain β-receptors which are responsive to noradrenaline and blocked by dichloroisoprenaline (Kern, 1970). The circular iris muscle of the cat is also relaxed by tyramine, an indirectly-acting amine (Kern, 1970). These responses are also blocked by a β-receptor antagonist.

The present experiments showed that responses to noradrenaline, mediated through the β-receptors of the bovine iris, are sensitized considerably by blockade of extraneuronal uptake. The shifts of 1.5 and 2.1 in the dose-ratios, by cocaine and the steroid, respectively, if taken to represent effects entirely on terminating processes (which is unlikely), mean that 34% and 52% of the agonist is inactivated by neuronal and extraneuronal uptake respectively. Combined blockade of both routes caused a nearly additive effect on the blockade of inactivation capacity.

The results obtained from tracheal muscle, where adrenergic responses are inhibitory, are similar to those described for the iris sphincter. Shifts in the

ED_{50} ratios of 2.2 and 3.2 after cocaine and 17β-oestradiol represent apparent reductions in the inactivation capacity of 55% and 69% respectively. Combined treatment with both inhibitors gave an even greater effect, as expected when mechanisms which operate essentially independently are simultaneously eliminated. The overlap in the effects of the two inhibitors is likely to be an expression of the action of at least one of them on access pathways rather than solely on terminating processes (Kalsner, 1976). This is more obvious when the findings with the facial artery are described. Although the innervation of the facial artery was not studied, the facial vein of the rabbit responds both to sympathetic nerve stimulation and to noradrenaline with relaxation (Pegram, Bevan & Bevan, 1976) and in the present experiments, relaxation was observed routinely over a wide concentration-range of the catecholamine. The striking shifts of 20 and 44 in the ED_{50} ratios after the steroid and cocaine imply separate reductions of 95 and 98% in the inactivation capacity of the tissue for noradrenaline, and obviously cannot simply be due to independent effects on termination of action but involve effects on access barriers and perhaps other processes.

Examination of the uptake of [^{3}H]-noradrenaline, in the presence of cocaine to block neuronal transport, showed that the accumulation of radioactivity was diminished significantly during exposure to 17β-oestradiol, confirming a cocaine-insensitive site of action for the steroid (Kalsner, 1969), in all three preparations. The quantitative relationship between the percentage inhibition of total uptake and the amount of response sensitization which should be correspondingly anticipated cannot be defined. This is so since the actual fraction of the total tissue uptake which represents transport into effector cells cannot be ascertained, nor is the location and kinetic conditions of these transport sites with respect to the β-receptors known. This information is necessary if effects on uptake are to be correlated successfully with response magnitudes (Kalsner, 1977).

The role of extraneuronal uptake as an inactivation process as determined by an analysis of response magnitudes is significant, although reliable quantification of its contribution to termination of action is not now possible.

Belfrage, Fredholm & Rosell (1977) and Belfrage (1978) observed that inhibition of catechol-*O*-methyltransferase enhanced noradrenaline-induced vasodilatation in adipose tissue and speculated that there was a differential distribution of α- and β-receptors in vascular and perhaps other tissues such that the β-receptors were remote from neuronal uptake sites and subsidiary to the innervated and dominant α-receptors. However, the present findings were made in preparations in which the β-receptor predominates and gives the organ its characteristic response to noradrenergic stimuli. Interestingly, in support of the present interpretation, Belfrage and collaborators (1977; 1978) noted that inhibition of *O*-methylation increased by 50%, the lipolytic response in an *in situ* dog preparation, whether it was induced by sympathetic nerve stimulation or by noradrenaline. The β-receptors are the dominant adrenoceptors in the fat cells mediating lipolysis in the dog (Belfrage *et al.*, 1977).

The present observations show a sensitization of β-receptor-mediated responses by an inhibition of extraneuronal uptake in three distinct smooth muscle preparations in cattle, even when the neuronal uptake sites are apparently fully functional. Thus, the β-adrenoceptors are located sufficiently close to the extraneuronal uptake sites for the latter to be exceedingly influential in affecting the concentration and duration of the catecholamine at its sites of action. These findings extend the earlier data on coronary arteries. They point more clearly to a role for extraneuronal mechanisms of inactivation in the termination of responses to noradrenaline than could be reached by simple comparative quantitative estimations of [^{3}H]-noradrenaline uptake, after neuronal and extraneuranol blockade by inhibitors of each process (Kalsner, 1976; 1977).

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