concentrated to give samples having >1·0 mg/ml protein. The fractions at the protein peak of each secretion were also combined and concentrated to give samples having similar concentrations of protein.

The concentrated combined fractions were separately dialysed against 0·01M sodium acetate buffer, pH 3·6 in 0·15M NaCl and the interaction with sodium salicylate at a final concentration of 100 mM was measured turbidimetrically as described. The results are shown in Table 1.

Neither the glycoprotein nor the protein fractions from either sample of post-histalog gastric juice interacted with sodium salicylate, confirming the observations with unfractionated post-histalog gastric juice. However, protein fractions of both resting gastric juice and saliva interacted strongly with sodium salicylate. There was also a very slight interaction with the glycoprotein fraction in both resting gastric juice and saliva.

Although little precipitation of the separated glycoproteins of resting gastric juice or saliva was detected, it is possible that in the whole secretions there may be some coprecipitation of the glycoprotein during precipitation of the protein.

However, to obtain any detectable precipitation of the protein from either gastric juice or saliva the secretion had to be concentrated at least 10 times, and even in the concentrated secretions, the maximum precipitation obtained represented less than 20% of the total protein and glycoprotein. Thus it seems unlikely that in man there would be any significant precipitation of glycoprotein of gastric juice by 100 mM sodium salicylate *in vivo*.

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Effects of desipramine, phentolamine and phenoxybenzamine on the release of noradrenaline from isolated tissues

Adrenergic nerves in isolated tissue incubated with [^{3}H]noradrenaline (^{3}H-NA) take up the amine by an active mechanism, the membrane pump, and incorporate it into the amine storage granules (Carlsson 1966; Hamberger 1967; Jonsson, Hamberger & others, 1969). Field stimulation of isolated tissue is known to cause release of ^{3}H-NA from the adrenergic nerves (Baldessarini & Kopin 1967; Farnebo & Hamberger 1970). The effects of membrane pump blocking and α-receptor blocking drugs on transmitter release and overflow have been examined in several experimental models with divergent results (see e.g. Brown & Gillespie, 1957; Blakeley, Brown & Ferry, 1963; Thoenen, Huerlimann & Haefely, 1964a, b; Boullin, Costa & Brodie, 1967). Field stimulation of isolated tissue was considered an appropriate model for such studies as the stimulation did not affect the circulation in the tissue. We now report the influence of drugs on the ^{3}H-NA release from central and peripheral tissues.

Isolated irides and cerebral cortex slices of standardized size (diameter 3 mm, thickness 0·5 mm) from untreated female rats (Sprague–Dawley, 180–200 g) were carefully prepared. The tissue was incubated at 37° in a modified Krebs-Ringer bicarbonate

medium containing 10^{-7}M ^{3}H-(±)-NA (10 Ci/mmol, New England Nuclear) (see Jonsson & others 1969). After 30 min incubation it was transferred to small stimulation chambers and superfused with ^{3}H-NA-free buffer containing the drug to be tested. After superfusion for 30 min the tissue was stimulated by an electric field (biphasic pulses, 12 mA, 2 ms, 10/s) (Farnebo & Hamberger 1970) for 10 (iris) or 2 (cortex) min and superfused for another 15 min. The superfusate (0·5 ml/min) was collected in 5 min fractions and analysed for total radioactivity by liquid scintillation counting. After the superfusion the tissue was dissolved in Soluene and analysed for total radioactivity. Quenching was measured by re-counting representative samples after the addition of a standard amount of [^{3}H]toluene. The stimulus-induced release was measured as total tritium overflow during the stimulation minus the calculated spontaneous overflow during the same period, and was expressed as per cent of the tritium content in the tissue at the onset of stimulation (calculated by adding the tritium efflux and the tritium content of the tissue at the end of the superfusion). The following drugs were tested: desipramine 10^{-8} — 10^{-6}M; phentolamine 10^{-6} — 10^{-5}M; phenoxybenzamine 10^{-6} — 10^{-5}M.

Field stimulation caused release of radioactivity both from isolated irides and from cerebral cortex slices (see Table 1). This release has been shown to consist mainly of unchanged ^{3}H-NA (Baldessarini & Kopin 1967; Häggendal, Johansson & others, 1970). The fraction of the exogenous ^{3}H-NA overflowing per impulse was calculated to be $2{\cdot}0 \times 10^{-5}$ for iris and $12{\cdot}7 \times 10^{-5}$ for cerebral cortex. Desipramine which is a potent inhibitor of the membrane pump in noradrenaline nerves (Hamberger 1967), caused a rather small increase of the overflow in both tissues. The α-receptor blocking drugs phentolamine and especially phenoxybenzamine 10^{-6}M caused a larger increase of the overflow than desipramine. In irides, phenoxybenzamine 10^{-5}M further increased the overflow to about four times the control. We have found phenoxybenzamine 10^{-5}M, but not 10^{-6}M, to effectively block the membrane pump in isolated irides, and this may explain the present difference between 10^{-5} and 10^{-6}M phenoxybenzamine. In agreement with this assumption the overflow with phenoxybenzamine 10^{-6}M could be augmented by desipramine (Table 1). Also, the increase

Table 1. *The effect of drugs on field stimulation induced release of* [^{3}H]*noradrenaline.* Isolated irides or cerebral cortex slices from untreated rats were incubated with ^{3}H-NA 10^{-7}M, and then superfused for 30 min with ^{3}H-NA-free buffer to which the drugs to be tested had been added. Subsequently the tissue was stimulated for 10 (iris) or 2 (cortex) min and after rinsing for 15 min the radioactivity in the tissue was determined. The stimulation induced overflow of tritium is expressed as per cent of the tritium content in the tissue at the onset of the stimulation (see text) and the values are given as mean ± s.e. Number of observations within brackets.

Drug	Concentration M	Iris	Cerebral cortex
None		11·8 ± 0·5 (37)	15·1 ± 0·9 (28)
Desipramine	10^{-8}	12·3 ± 1·3 (7)	18·2 ± 1·3 (8)
Desipramine	10^{-7}	16·5 ± 1·2 (18)	17·2 ± 1·1 (7)
Desipramine	10^{-6}	18·9 ± 1·0 (11)	20·5 ± 1·9 (7)
Phenoxybenzamine	10^{-6}	32·6 ± 3·2 (15)	26·8 ± 1·7 (8)
Phenoxybenzamine	10^{-5}	49·9 ± 2·8 (12)	25·5 ± 2·3 (6)
Phentolamine	10^{-6}	20·5 ± 0·5 (4)	26·7 ± 2·0 (7)
Phentolamine	10^{-5}	20·2 ± 1·7 (8)	22·5 ± 1·8 (8)
Desipramine + phenoxybenzamine	10^{-7} 10^{-6}	42·1 ± 2·5 (12)	
Desipramine + phentolamine	10^{-7} 10^{-5}	29·8 ± 2·1 (6)	

induced by phentolamine could be potentiated by desipramine. The increased overflows caused by desipramine in combination with α-receptor blocking drugs were in fact larger than the increase caused by desipramine alone.

It has been claimed that phenoxybenzamine increases overflow of noradrenaline by blocking its binding to the α-receptors (Brown & Gillespie, 1957, Boullin & others, 1967). But we have found phenoxybenzamine to cause a markedly increased noradrenaline overflow also in the mouse isolated atrium and a direct effect of this α-blocking drug on the adrenergic nerves, not related to membrane pump inhibition, can thus not be excluded. The present findings might also be explained by an interaction between the adrenergic nerves and the α-receptors which could lead to a variable release (Häggendal 1970). Thus, membrane pump blockade could be expected to cause a marked increase of the overflow as the transmitter is not readily taken up again. However, as the overflow is only slightly increased, it may be assumed that the release from the adrenergic nerves is decreased after inhibition of the membrane pump. When the α-receptors are blocked there is a marked increase of the overflow suggesting that the receptor blockade led to an increased release of the transmitter from the adrenergic nerves, compared to the control. Combination of membrane pump and α-receptor blockade caused the most marked increase of the transmitter overflow but the effect on the release is unclear. Thus, in agreement with Häggendal (1970) the receptor organ seems to be able to influence the amount of transmitter released.

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