It seems, therefore, that stabilization of the erythrocyte membrane by anti-inflammatory drugs is probably due to a stabilizing effect of the drugs on some proteins in the cell membrane in a manner similar to that observed by us in the experiments on protein denaturation (Mizushima, 1968).

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Inhibition of the noradrenaline uptake in guinea-pig vas deferens by continuous nerve stimulation

The release and the uptake of noradrenaline by nerve terminals can be modulated by different regulatory mechanisms. Gillis (1963) observed that short-lasting stimulation of sympathetic nerves increased the uptake of noradrenaline by the cat perfused heart. Blakeley & Brown (1964) found that nerve stimulation produced an inhibition of the uptake of noradrenaline infused into the cat spleen. Chang & Chiueh (1968) demonstrated that intermittent nerve stimulation of the branches of the facial nerve increased the uptake of tritiated noradrenaline (^{3}H-NA) in rat submaxillary gland. We now report how continuous nerve stimulation with different frequencies can affect the noradrenaline uptake. The hypogastric nerve-vas deferens preparation was chosen because of the abundance of adrenergic synapses in this organ and the opportunity it offers for pre- and postganglionic stimulation (Birmingham & Wilson, 1963).

Male guinea-pigs of about 400 g provided the vasa deferentia and hypogastric nerve preparations which were incubated in a 50 ml bath in modified Krebs solution (Huković, 1961) at 37° for 30 min. The vas deferens from one side was stimulated while that on the other side served as control. The electrical stimulation of the hypogastic nerve was performed as described by Huković (1961), and transmural stimulation as described by Birmingham & Wilson (1963). Monophasic square stimuli of supramaximal strength and 1 ms duration were applied continuously for 30 min. The contractions of the vas deferens were recorded on smoked paper. For the transmural stimulation the vas deferens was dissected and cleaned of peritoneum and surrounding fat tissue.

^{3}H-NA (specific activity 190 mCi/mg) was added to the bath (final concentration, 5 ng/ml of medium). According to Avakian & Gillespie (1968) this concentration is low enough to ensure the uptake of noradrenaline exclusively by the nerve terminals. Oxidation of ^{3}H-NA was prevented by the addition of EDTA (10 mg/litre) and ascorbic acid (20 mg/litre). The pH of the medium was 7·4. After incubation, the preparations were washed twice in 5 ml of saline for 30 s and blotted dry on filter paper. The tissue was homogenized in 0·4N perchloric acid. ^{3}H-NA was separated on alumina columns (Whitby, Axelrod & Weil-Malherbe, 1961). The [^{3}H]radioactivity was measured by a Packard liquid scintillation counter. The amount of ^{3}H-NA metabolites was calculated by subtracting the specific ^{3}H-NA radioactivity from the total [^{3}H]radioactivity.

Low frequency (1 and 6/s) pre- and post-ganglionic stimulation produced 34–44% inhibition of the ^{3}H-NA uptake (Table 1). High frequency (50/s) reduced the uptake by 90–95%. The amount of ^{3}H-NA metabolites varied, also, with the frequency of stimulation; the highest level being observed after 6/s postganglionic stimulation. After 1 and 50/s, the level of ^{3}H-NA metabolites was somewhat lower or did not differ from control value.

Table 1. *Effect of continuous nerve stimulation on the ^{3}H-NA uptake. The results are expressed in* nCi/g *tissue and* P *values show the degrees of significance between control and stimulated preparations. Mean of* 4–6 *experiments*

	^{3}H-NA		^{3}H-NA-metabolites	
	Preganglionic	Postganglionic	Preganglionic	Postganglionic
Control	319·89 ± 15·92	303·50 ± 8·52	20·42 ± 5·36	27·41 ± 15·06
1/s	198·94 ± 19·91 $P < 0.005$	—	15·21 ± 1·75 n.s.	—
6/s	181·00 ± 13·59 $P < 0.001$	201·53 ± 18·75 $P < 0.001$	67·83 ± 11·08 $P < 0.005$	92·72 ± 17·41 $P < 0.01$
50/s	34·02 ± 3·25 $P < 0.001$	17·52 ± 10·11 $P < 0.001$	52·86 ± 16·10 $P < 0.05$	28·73 ± 8·13 n.s.

Our results show that the uptake of ^{3}H-NA is impaired by continuous preganglionic and postganglionic stimulation. This effect was frequency-dependent. One possible explanation for the observed effect is to assume that the depolarization of the neuronal membrane produced by nerve stimulation inhibits the re-uptake and favours the release of noradrenaline. Therefore, at high frequency the inhibiting effect on the uptake process becomes more evident because the depolarization lasts longer. If this were true, then the release and the re-uptake are not simultaneous but sequential processes for a certain area of the synaptic membrane. The alternative explanation could be the existence of a concentration barrier in the synaptic cleft in which a high concentration of endogenous noradrenaline released by high frequency stimulation exceeds the capacity of the re-uptake mechanism and blocks the access of ^{3}H-NA to the synaptic membrane. Recently, Malmfors (personal communication), using a histochemical fluorescence technique, observed a similar effect of nerve stimulation on the uptake of noradrenaline. In the light of these results a reconsideration of the role of the re-uptake mechanism in adrenergic neurotransmission cannot be avoided. It appears that this mechanism has a limited capacity and does not occur simultaneously with the release process at certain areas of the synaptic membrane.

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