Interaction between indomethacin, oxymetazoline and phentolamine on the release of [^{3}H]noradrenaline from brain slices

Recent evidence indicates that local feed-back mechanisms regulate the amount of noradrenaline released upon stimulation from peripheral and central neurons. On the one hand, liberated noradrenaline depresses the secretory response to forthcoming nerve impulses by an action on α-adrenoceptors; exogenous α-adrenoceptor stimulant drugs mimic this effect, while α-adrenoceptor blocking agents interrupt the feed-back loop and enhance the liberation of noradrenaline (Farnebo & Hamberger, 1971 a, b; Starke, 1971, 1972; Starke & Montel, 1973 b). On the other hand, prostaglandins are released during sympathetic nerve activity from unknown sources and decrease the liberation of noradrenaline; inhibition of their biosynthesis enhances the secretory response to stimulation (Hedqvist, 1970; Swedin, 1971; Chanh, Junstad & Wennmalm, 1972; Junstad & Wennmalm, 1972; Bergström, Farnebo & Fuxe, 1973).

It is tempting to consider that these two mechanisms might be coupled: extracellular noradrenaline might release prostaglandins by an activation of α-adrenoceptors, and the lipids might ultimately interfere with transmitter secretion (Swedin, 1971). The present experiments were done to test this possibility in central neurons. Rat brain slices were preincubated with [^{3}H]noradrenaline. The effect of oxymetazoline (α-adrenoceptor stimulant) and phentolamine (α-adrenoceptor blocking agent) on the efflux of tritium was evaluated, after the synthesis of prostaglandins had been blocked by indomethacin (Flower & Vane, 1972). The stimulation-induced overflow of tritium is a good measure of the release of [^{3}H]noradrenaline (Baldessarini & Kopin, 1967).

Results are summarized in Table 1. None of the agents altered the *spontaneous*

Table 1. *Interaction between indomethacin, oxymetazoline and phentolamine on the stimulation-induced overflow of tritium from brain slices preincubated with* [^{3}H]*noradrenaline.*

Drugs in superfusion fluid (M)	% Stimulation-induced tritium overflow	
	Control group[a]	Indomethacin group[b]
—	3·86 ± 0·18 (10)	5·05 ± 0·52* (10)
Oxymetazoline 10^{-5}	1·65 ± 0·30** (8)	1·57 ± 0·26** (9)
Phentolamine 10^{-6}	12·06 ± 1·25** (10)	14·54 ± 1·41** (10)
Oxymetazoline 10^{-5} + phentolamine 10^{-6}	1·75 ± 0·23** (8)	1·78 ± 0·36** (10)

Slices of the rat cerebral cortex, diameter about 3 mm, were preincubated with 10^{-7} M (—)-[^{3}H]-noradrenaline, 6·4 Ci mmol^{-1}, for 30 min. They were then superfused with fresh Krebs-Ringer solution, and after superfusion for 30 min stimulated by an electrical field for 2 min (duration of pulses 2 ms, 12 mA, 5 Hz). The stimulation-induced tritium overflow was calculated as % of the tritium content of the slices at the onset of stimulation.

[a] Rats were given a daily injection of indomethacin solvent (see below) for the three days before the experiment; no indomethacin in the superfusion fluid.

[b] Rats were given a daily s.c. injection of 5 mg kg^{-1} indomethacin, dissolved in 0·5 ml of 0·1 M sodium phosphate buffer pH 7·2, for the three days before the experiment; $3·2 \times 10^{-5}$ M indomethacin in the superfusion fluid in addition to oxymetazoline or phentolamine. Means ± s.e.m.; number of experiments in brackets. Significant differences (*t*-test): * from the control group ($P < 0·05$); ** from experiments in the absence of oxymetazoline and phentolamine ($P < 0·001$).

outflow of radioactive material (not shown). In the absence of drugs with affinity for α-adrenoceptors, pretreatment plus superfusion with indomethacin slightly augmented the *stimulation-induced* overflow, indicating that endogenous prostaglandins modulate transmitter secretion from central noradrenergic neurons (*cf.* for peripheral tissues: Chanh & others, 1972). In brain slices from control rats oxymetazoline decreased, and phentolamine increased, the stimulation-induced overflow (Table 1). At the high concentration of 10^{-5} M, oxymetazoline prevented the effect of phentolamine (cf. Starke & Montel, 1973 b). Inhibition by indomethacin of the synthesis of prostaglandins failed to impair either the inhibitory effect of the α-adrenoceptor stimulant and the facilitatory effect of the blocking agent. It should be noted that, in rats, as little as 0·5 mg kg^{-1} of indomethacin promptly increased the urinary excretion of noradrenaline, presumably through blockade of prostaglandin biosynthesis (Junstad & Wennmalm, 1972). $3{\cdot}6 \times 10^{-6}$ M indomethacin inhibited the prostaglandin synthetase from rabbit brain by 50% (Flower & Vane, 1972). 10^{-5} to 7×10^{-5} M indomethacin abolished the outflow of the lipids from the rabbit heart (Chanh & others, 1972). Thus, it may be assumed that the combined pretreatment and superfusion with indomethacin used here strongly inhibited the formation of prostaglandins.

The persistence of the effects of oxymetazoline and phentolamine in the presence of indomethacin makes it unlikely that the regulation of transmitter release through the action of liberated noradrenaline on α-adrenoceptors requires prostaglandins as a chemical link. Recent experiments in peripheral tissues support this conclusion (Hedqvist, 1973; Starke & Montel, 1973 a; Stjärne, 1973). Both in peripheral and central noradrenergic neurons the control of transmitter release apparently operates via two independent feed-back loops: one, mediated by prostaglandins, the other, by the action of noradrenaline on (possibly prejunctional) α-adrenoceptors.

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