Regulation of [^{3}H]5-hydroxytryptamine release from rat brain slices

Many psychoactive drugs are considered to exert their action through interference with monoaminergic transmitter mechanisms. 5-Hydroxytryptamine (5-HT) neurons are widely distributed throughout the brain (Fuxe, Hökfelt & Ungerstedt, 1968) and release of 5-HT has been demonstrated in the brain (Aghajanian, Rosecrans & Sheard, 1967; Chase, Katz & Kopin, 1969; Farnebo, 1971).

In studies on release of noradrenaline from isolated peripheral tissues it was shown that α-adrenoceptor stimulation decreases noradrenaline release while α-adrenoceptor blockade increases noradrenaline release (Farnebo & Hamberger, 1970, 1971a). β-Adrenoceptor stimulation or blockade does not influence noradrenaline release (Werner, Wagner & Shümann, 1971; Farnebo & Hamberger, 1974). The feed-back regulation of noradrenaline release from adrenergic nerves has been suggested to be mediated via presynaptic receptors (Farnebo & Hamberger, 1971a; Starke, 1971; Enero, Langer & others, 1972). The same may also apply to central monoamine nerve terminals (Farnebo & Hamberger, 1971b). Starke & Montel (1973) have proposed that transmitter release from central noradrenaline as well as 5-HT neurons can be modulated via activation of presynaptic α-adrenoceptor sites. In order to further investigate release and release regulation in 5-HT nerve terminals, field stimulation of brain slices was made (Chase & others, 1969). The aim was to find out whether 5-HT release is controlled by the same mechanism as noradrenaline release, i.e. via influence on presynaptic noradrenaline receptors (α-adrenoceptors) located on the 5-HT nerve terminals or if 5-HT release is likely to be controlled by 5-HT itself via presynaptic 5-HT receptors.

Coronal brain slices about 0·5 mm thick were prepared from female albino Sprague-Dawley rats. Round pieces, 3 mm in diameter were punched out and preincubated with ^{3}H-5-HT ([^{3}H] 5-hydroxytryptamine-^{3}(G)-creatinine sulphate, 5–10 Ci mmol^{-1}, New England Nuclear), 10^{-7}M for 30 min. After rinsing for a few seconds the tissue was transferred to a superfusion chamber and superfused with buffer for 30 min before stimulation. Two 2 min stimulations (10 Hz, 2 ms, 12 mA, biphasic pulses) were applied with a 15 min interval. During the second period the drug to be tested was present in the buffer. The tissue was then solubilized in Soluene and total radioactivity determined in tissue and superfusion medium using a liquid scintillation counter. The stimulation-induced overflow of radioactivity was determined and the overflow during the second stimulation period expressed as per cent of that during the first one. For further details on the experimental procedure see Farnebo (1971), Farnebo & Hamberger (1971b).

The monoamine oxidase inhibitor nialamide did not change the stimulation-induced overflow of 5-HT (Table 1).

Chlorimipramine caused a strong increase of 5-HT overflow due to its blockade of the 5-HT membrane pump. Methiothepin, claimed to be a 5-HT receptor blocking agent (Monachon, Burkard & others, 1972), significantly increased the stimulation-induced overflow. Since methiothepin does not inhibit 5-HT uptake (unpublished) it is probable that the increased overflow is due to an increased release of 5-HT. A significantly decreased release was caused by ergocornine, which *in vivo* has been found to be a central 5-HT receptor stimulating agent (Corrodi, Fuxe & others, 1973; Fuxe, Corrodi, Farnebo & Hamberger, to be published). Another ergot drug, 2-brom-α-ergocryptine (CB154), which *in vivo* has little 5-HT receptor stimulating activity, only at a high concentration (10^{-5}M) depressed 5-HT release.

Table 1. *Drug-induced changes of tritium overflow during field stimulation of brain slices preincubated with ^{3}H-5-HT (for details see text).*

Drug	Concentration (M)	Overflow in % of 1st stimulation	Significance[1]
Buffer	—	80 ± 3 (42)	
Nialamide	10^{-7}	86 ± 6 (6)	NS
	10^{-6}	87 ± 8 (6)	NS
Chlorimipramine	10^{-7}	108 ± 6 (6)	xx
	10^{-6}	157 ± 20 (6)	xxx
Methiothepin	2×10^{-7}	75 ± 5 (6)	NS
	2×10^{-6}	112 ± 11 (12)	xxx
Ergocornine	10^{-7}	82 ± 9 (11)	NS
	10^{-6}	61 ± 7 (9)	xx
2-Brom-α-ergocryptine	10^{-7}	71 ± 3 (6)	NS
	10^{-6}	71 ± 9 (6)	NS
	10^{-5}	59 ± 9 (5)	x
Clonidine	10^{-7}	83 ± 5 (6)	NS
	10^{-6}	79 ± 6 (18)	NS
Phentolamine	10^{-7}	78 ± 9 (5)	NS
	10^{-6}	80 ± 5 (12)	NS
	10^{-5}	108 ± 9 (15)	xx
Apomorphine	10^{-7}	80 ± 4 (6)	NS
	10^{-6}	76 ± 5 (6)	NS
Pimozide	10^{-7}	85 ± 3 (6)	NS
	10^{-6}	98 ± 6 (15)	x

[1] Significance of differences between buffer and drug: x $P<0.05$; xx $P<0.01$; xxx $P<0.001$ (Student's *t*-test).

In previous studies LSD, which stimulates central 5-HT receptors, has been shown to depress 5-HT release (Katz & Kopin, 1969; Farnebo & Hamberger, 1971b). The noradrenaline and dopamine receptor stimulating agents clonidine and apomorphine respectively which previously were found to decrease catecholamine release (Farnebo & Hamberger, 1971b) did not affect the stimulation-induced overflow of 5-HT. The noradrenaline receptor blocking agent phentolamine increased the 5-HT overflow. However, the concentration of phentolamine needed was higher than necessary to affect noradrenaline overflow. Also the dopamine receptor blocking agent pimozide increased 5-HT overflow. In an earlier study no effect of chlorpromazine on 5-HT overflow was obtained (Farnebo & Hamberger, 1971b).

Starke & Montel (1973) have recently shown that a high concentration (10^{-5}M) of clonidine can depress 5-HT release, and they concluded also that 5-HT neurons may be endowed with receptor structures similar to the α-adrenoceptor. However, the receptors on the 5-HT nerve terminals are less sensitive to clonidine than those of noradrenaline neurons. Also, the catecholamine receptor blocking drugs are less potent on 5-HT overflow. Furthermore, the findings that ergocornine depresses and methiothepin increases 5-HT release point to the possibility that 5-HT release can be modified by 5-HT acting on presynaptic 5-HT receptors. Thus, the present results support the idea (Farnebo & Hamberger, 1971b, 1973) that transmitter release from central monoamine neurons can be modulated by the transmitter of the neuron.

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Department of Histology,
Karolinska Institutet,
S-104 01 *Stockholm, Sweden.*

L.-O. FARNEBO
B. HAMBERGER

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Influence of α- and β-adrenoceptors on the release of noradrenaline from field stimulated atria and cerebral cortex slices

Noradrenaline release from peripheral and central noradrenaline nerves can be influenced by drugs affecting α-adrenoceptors (Häggendal, 1970; Farnebo & Hamberger, 1970, 1971a, b; Starke, 1971; Kirpekar & Puig, 1971; Enero, Langer & others, 1972; Starke & Altmann, 1973). Stimulation of α-adrenoceptors depresses noradrenaline release while blockade of α-adrenoceptors increases it. The release of noradrenaline is under physiologic conditions probably controlled by the synaptic concentration of the amine. It has been suggested that this feed-back control of noradrenaline release is mediated via presynaptic α-adrenoceptors, because drugs affecting α-adrenoceptors have been found to be effective also in heart tissue where the adrenoceptor of the effector cell is of the β type (Farnebo & Hamberger,1971a; Starke, 1971; Enero & others, 1972; Starke & Altmann, 1973).

β-Adrenoceptors seem to be of small significance in the regulation of noradrenaline release in the guinea-pig heart (Werner, Wagner & Shümann, 1971), while little is known about the role of β-adrenoceptors in the regulation of noradrenaline release in the brain. The aim of this investigation was to study the release of noradrenaline from field stimulated mouse atria and rat cerebral cortex slices and to compare the effect of α- and β-adrenoceptor stimulating and blocking drugs on this release.

Mouse isolated atria (N.M.R.I.; weight about 2–3 mg) or rat cerebral cortex slices (Sprague-Dawley; weight about 5 mg) were incubated with (±)-[7-^{3}H] noradrenaline (^{3}H-NA) (5–10 Ci mmol^{-1}, Radiochemical Centre, Amersham) 10^{-7}M for 30 min at 37°. Single atria or slices were then superfused by Krebs-Ringer bicarbonate buffer to which the drug to be tested was added. After 30 min superfusion the tissue was stimulated by an electrical field (10 Hz, 12 mA, 2 ms, biphasic pulses) for 10 min (atria) or 2 min (cortex slices) and then further superfused for 15 or 13 min respectively (Baldessarini & Kopin, 1967; for details see Farnebo & Hamberger, 1970, 1971a,b). Total radioactivity in the superfusate and tissue was