Evidence *in vitro* for a 5-HT link in dopaminergic neurotransmission in the anterior hypothalamic region of the rat: demonstration of a 5-HT link in dopaminergic thermoregulation?

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Both dopamine (DA) (Cox, Kerwin & Lee, 1978) and 5-hydroxytryptamine (5-HT) (Lin, 1978) may have a physiological role in heat loss mechanisms in the rat. Furthermore it has been suggested that DA-dependent heat loss mechanisms are mediated through a 5-HT link in the rat hypothalamus (Maj, 1977). We have investigated this interaction *in vitro* using two approaches. Firstly we have studied the effect of dopamine and the dopamine receptor agonist apomorphine, in combination with various neuroleptic drugs, on the efflux of radioactivity from superfused anterior hypothalamic slices (0.2 × 0.2 mm) prelabelled with 10^{-7} M [^{3}H]-5-HT. The methods have been described in detail elsewhere (Kerwin & Pycock, 1979). Secondly, we have investigated the DA-sensitive adenylate cyclase in crude membranes prepared from the anterior hypothalamic region of rats which one week previously had received either a bilateral stereotaxic injection of the 5-HT neurotoxin, 5,6-dihydroxytryptamine (5,6-DHT, 10 μg/2 μl) or 2 μl of 0.1% ascorbic acid into the anterior hypothalamus.

In release studies a depolarizing stimulus (50 mM KCl) stimulated the efflux of radioactivity from superfused slices of hypothalamic tissue preloaded with [^{3}H]-5-HT: this effect was reduced in the absence of calcium (2 mM $MgCl_2$). Moreover the hypothalamic slices accumulated [^{3}H]-5-HT with apparent high affinity (K_m = 1.37 μM, 5 mg tissue, 10 min incubation, 0.2–2 μM [^{3}H]-5-HT); suggesting that the tissue slices contain functionally intact terminals for 5-HT. Both DA (>50 μM) and apomorphine (>200 μM) stimulated the spontaneous efflux of [^{3}H]-5-HT. The effects of DA (500 and 250 μM) and apomorphine (300 and 600 μM) were abolished in the presence of haloperidol (2 μM). Furthermore (+)-butaclamol but not (−)-butaclamol (both at 1 μM) abolished the effects of DA (250 μM) and apomorphine (300 μM). As a regional control, similar experiments were performed in hippocampal slices which possess 5-HT but not DA containing terminals (Ungerstedt, 1971). [^{3}H]-5-HT was released by 50 mM KCl in a calcium dependent fashion, but apomorphine and DA (both up to 500 μM) were without effect.

When incubated with crude membrane preparations from anterior hypothalamus DA (5–50 μM) stimulated adenylate cyclase activity in vehicle injected rats but was without significant effect in 5,6-DHT lesioned rats. This lesion was associated with a 65% reduction in high affinity uptake of [^{3}H]-5-HT.

These results suggest that within the rat hypothalamus, DA receptors are present on 5-HT neurones, activation of which stimulates the release of 5-HT within this region, thus providing a correlation *in vitro* to the behavioural demonstration of a 5-HT link in dopaminergic thermoregulation in the rat.

R.W.K. is an M.R.C. student.

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Effects of some amino acids on K^+-induced release of [^{3}H]-DA from rat striatal tissue

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A number of studies in the literature have described the effects of amino acids on striatal dopamine (DA) release, sometimes with conflicting results. In particular, reports of the action of GABA on DA release from striatal slices have varied from no effect (Stoof & Mulder, 1977) to facilitation of basal or K^+-induced DA release (Giourgueff, Kemel, Glowinski & Besson, 1978; Kerwin & Pycock, 1979; Starr, 1977). Effects of other amino acids such as glycine and glutamate have also been described (Kerwin & Pycock 1979; Anderson & Roberts, 1978). We have investi-

gated the effects of several 'inhibitory' amino acids on stimulus-induced DA release as distinct from basal efflux.

Methods were as described previously (Martin & Mitchell, 1979). Briefly, small prisms of striatal tissue were suspended in physiological medium and incubated with [^{3}H]-dopamine to allow high affinity uptake. Tissue was then loaded onto filters in chambers kept at 37°C and continuously superfused with medium. Fractions of the effluent were collected and counted for radioactivity. After several minutes, when a steady rate of basal efflux was reached, more medium was added. This was either normal or with 15 mM K^+ (a submaximal pulse) and with the compounds under study added.

Under these conditions, we were unable to show any effect of GABA (10^{-5} M to 10^{-3} M) on either basal or K^+-induced release. Furthermore, bicuculline methiodide (10^{-5} M to 10^{-4} M) showed no effect on K^+-induced release, suggesting that the control K^+ pulse did not represent an already maximal facilitation of DA release by endogenous GABA. However, glycine did show a marked (concentration-dependent) facilitation of K^+-induced DA release without effect on basal efflux. The threshold for the effect was between 10^{-5} M and 10^{-4} M with a 4-fold facilitation at 10^{-3} M. Taurine and β-alanine (which often show properties intermediate between GABA and glycine) gave a small but significant facilitation of K^+-induced release only at 10^{-3} M. The facilitation of K^+-induced DA release by glycine (3.10^{-4} M) could not be blocked by strychnine (10^{-4} to 10^{-3} M). (Strychnine-resistant effects, thought to be mediated by glycine have been described in spinal cord (Ryall, Piercey & Polosa, 1972).) The glycine effect of DA release was however completely blocked by picrotoxinin (10^{-4} M) or by replacement of 87% of the Cl^- in the medium with the impermeant anion isethionate, and also by 10^{-4} M bicuculline methiodide. These results suggest the existence of a strychnine-insensitive receptor for glycine on DA terminals, capable of modulating DA release, and further that this effect of glycine on DA release is dependent on Cl^- and can be modified by a bicuculline-sensitive mechanism.

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Regional changes in brain dopamine receptor function during six months trifluoperazine administration to rats

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During 12 months continuous administration of neuroleptic drugs to rats striatal dopamine (DA) receptors become supersensitive (Clow, Jenner, Theodorou & Marsden, 1979; Clow, Jenner & Marsden, 1979). We now report changes in DA receptor activity in striatal, mesolimbic and mesocortical DA containing areas of brain during 6 months administration of trifluoperazine hydrochloride (TFP; 2.8–4.0 mg kg^{-1} day^{-1} p.o.) to male Wistar rats.

TFP administration for 1 month caused inhibition of apomorphine (0.5 mg/kg sc)—induced stereotyped behaviour, which disappeared by 3 months to be replaced by an exaggerated response to apomorphine after 6 months drug intake (stereotypy scores: TFP group 3.38 ± 0.22; control group 2.50 ± 0.14; $P < 0.05$).

Dopamine (1–150 μM) stimulation of striatal adenylate cyclase activity was inhibited 1 and 3 months after beginning TFP administration (stimulation caused by 50 μM DA being 39% and 60% respectively) of control values at these times; $P < 0.05$). After 6 months drug administration DA stimulation of striatal adenylate cyclase was enhanced (stimulation caused by 50 μM DA being 144% of control values; $P < 0.05$).