Interactions of putative neurotransmitters in the region of the raphé nuclei of the rat

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There is increasing evidence to suggest that neurotransmitter release in the brain is subject to control by other locally released transmitters (e.g., Reubi, Iversen & Jessell, 1977). We have examined the possibility that synaptic transmission in the 5-hydroxytryptamine (5-HT)-containing cell bodies of the raphé nuclei may be subject to similar influences. We have studied the effect of putative neurotransmitters on the release of 5-HT and γ-aminobutyric acid (GABA) from this region. After removal of the brain, horizontal cuts were made, one just below the cerebral aqueduct and the other above the medial lemniscus, at the level of A350 (König & Klippel, 1963). The central 1 mm portion of brain was taken and chopped in two directions at 0.2 mm intervals; this region containing dorsal and median raphé cell groups. The subsequent methods used to study the release of preloaded radiolabelled transmitter from superfused tissue slices *in vitro* have been described in detail elsewhere (Kerwin & Pycock 1979). Amino-oxyacetic acid (10 μM) or pargyline (50 μM) were present to inhibit labelled transmitter metabolism where appropriate.

A depolarizing stimulus (20 and 50 mM KCl) stimulated the rate of efflux of [^{3}H]-5-HT and [^{3}H]-GABA from raphé slices. The effect of KCl was markedly reduced in a low calcium, magnesium-substituted medium. In addition, tissue slices accumulated these labelled transmitters with apparent high affinity kinetics (Km = 1.50 μM for [^{3}H]-5-HT and 9.58 μM for [^{3}H]-GABA; substrate concentrations of 0.2–2 μM for 5 min). These results suggest the tissue slices contain functionally intact nerve terminals for these transmitters. GABA (100 and 500 μM) stimulated the spontaneous efflux of [^{3}H]-5HT from raphé slices. The effect was blocked by picrotoxin (50 μM) but not by strychnine hydrochloride (1 μM). Other inhibitory amino acids, β-alanine, glycine and taurine (all at 1 mM) were without effect on [^{3}H]-5-HT efflux. The efflux of [^{3}H]-5-HT was also stimulated by substance P (SP) at 50 and 100 μM. L-Noradrenaline (NA) (0.2–1 mM) stimulated the efflux of [^{3}H]-GABA but not that of [^{3}H]-5-HT. Neither dopamine nor 5-HT influenced the efflux of [^{3}H]-GABA.

This data may provide insight into the neuronal interactions within the raphé nuclei. The demonstration of NA-stimulated GABA release complements the hypothesis of Gallagher & Aghajanian (1976) that adrenergic influence on the raphé is mediated indirectly via GABA interneurones. Secondly the effect of GABA on raphé cell firing (Gallagher & Aghajanian 1976) may be associated with a concomitant modulatory release of 5-HT from dendrites or terminals, within this region. Although the raphé possesses SP-containing terminals (Cuello & Kanazawa 1978), the possible functional relevance of SP-stimulated 5-HT release is not yet clear.

R.W.K. is an MRC student.

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Behavioural and neurochemical studies on the striatonigral GABA pathway

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We have recently shown that denervation of nigral γ-aminobutyric acid (GABA) receptors by destruction of the striatonigral GABA pathway results in an enhanced dopamine (DA)—independent contralateral rotational response to unilateral intranigral injections of the GABA agonist, muscimol and elevated nigral [^{3}H]-GABA binding; this apparent denervation supersensitivity is characterised by an increase in the number of high affinity [^{3}H]-GABA binding sites (Waddington & Cross, 1978a). In this study we describe interrelationships between various behavioural and neurochemical indices of the integrity of the striatonigral GABA pathway.

Using previously described techniques (Waddington & Cross, 1978a, b; Waddington & Crow, 1978), male Sprague-Dawley rats of 150–200 g were given unilateral intrastriatal injections of kainic acid (KA, 2.5 μg/1 μl saline) to destroy striatal perikarya. After 17 days rats were tested for rotational responses to challenge with the DA agonist apomorphine (0.25 mg/kg s.c.). At 21 days after the lesion animals were given unilateral intranigral injections of the GABA agonist muscimol (2 ng/1 μl saline) ipsilateral to the striatal KA lesion and rotational responses recorded. After a further 7 days (i.e. 28 days post-lesion) rats were killed and striatal and nigral tissue dissected out from frozen sections for assay of glutamic acid decarboxylase (GAD) activity and GABA concentration.

Rats with unilateral striatal KA lesions showed ipsilateral rotational responses to apomorphine, and these responses were correlated with depletions of nigral GABA in the lesioned hemispheres ($r = 0.796$, $P < 0.01$). This supports the suggestion (Garcia-Munoz, Nicolaov, Tulloch, Wright & Arbuthnott, 1977) that the striatonigral GABA pathway constitutes the striatal output system and indicates that the rotational response to apomorphine is a good index of the integrity of this system. These ipsilateral rotational responses to apomorphine were also significantly correlated with the contralateral rotational responses to intranigral muscimol ($r = 0.774$, $P < 0.05$). Similarly, contralateral rotational responses to intranigral muscimol were correlated with depletions of nigral GABA ($r = 0.665$, $P < 0.05$).

Depletions of nigral GABA were not related to decreases in striatal GAD activity ($r = 0.180$, N/S). Striatal GAD activity presumably reflects not only the integrity of the cell bodies of origin of the striatonigral GABA pathway but also that of striatal GABAergic interneurons, and therefore seems to be a poor index of striatonigral function.

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Central properties of α-allophanyl-α-allyl-γ-valerolactone (valofan)

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The hydroxylated barbiturate proxibarbal (5-allyl-5(β-hydroxypropyl) barbituric acid) is used prophylactically in the treatment of migraine. Proxibarbal rearranges in solution (and perhaps also by *in vivo* metabolism) to produce the lactone, valofan (α-allophanyl-α-allyl-γ-valerolactone) (Bobranski, Konieczny & Syper, 1962). Valofan may be responsible for the pharmacological actions on the parent drug (Hano, Trabka & Sieroslawska, 1968), but its central actions are unknown. The present study investigates biochemically and behaviourally the action of valofan, following acute administration, on cerebral dopamine and 5 hydroxytryptamine (5HT) mechanisms.

Administration of valofan (in doses of 0.5 to 1.5 g/kg ip) to mice (20–25 g) produced a decrease in exploratory behaviour progressing to loss of righting reflexes and unconsciousness. Administration of valofan (in doses up to 2.0 g/kg ip) to rats had similar effects, and inhibited the stereotyped behaviour induced by administration of amphetamine sulphate (5 mg/kg ip) or apomorphine hydrochloride (0.5 mg/kg sc). (ED_{50} 1.3 g/kg in each case). However, this effect was only observed at doses causing marked sedation.

Administration of valofan (1.5 g/kg ip) to rats elevated dopamine, HVA and DOPAC levels in both mesolimbic and striatal areas ($P < 0.05$). This effect was maximal at 3 h and thereafter declined so that HVA levels were markedly reduced in both brain regions at later time intervals (8 or 16 h) when compared with control animals ($P < 0.05$). Most of these effects had disappeared by 24 hours.

These effects on cerebral dopamine were not