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5-Hydroxytryptamine and 5-hydroxytryptaminergic-dopaminergic interactions in the ventral tegmental area of rat brain

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The ventral tegmental area (VTA) contains the somata and dendrites of A10 dopaminergic neurons (Phillipson 1979a), and lesions of this region produce profound behavioural changes which have been termed the ventromedial tegmentum or A10 syndrome (Le Moal et al 1977). Various anatomical studies have revealed that 5-hydroxytryptaminergic axons ascending from the dorsal raphe nucleus innervate this area (Taber-Pierce et al 1976; Bobiller et al 1976; Phillipson 1979b) and these neurons have recently been suggested to contribute to the behavioural deficits accompanying lesions of the VTA (Sessions et al 1980). The aims of the present study were to confirm the 5-hydroxytryptaminergic innervation of the VTA and to investigate the interaction between 5-HT and dopamine (DA).

5-Hydroxytryptaminergic innervation of the VTA

Male Sprague-Dawley rats (150–250 g) were used and tissue enriched in the VTA (average wet weight 7·3 mg) was dissected as previously described (Beart & Gundlach 1980). Slices of VTA (0·2 × 0·2 mm, equivalent to 2·5 mg of fresh tissue) were preincubated at 37 °C in a shaking water bath for 5 min and then incubated with [^{3}H]-5-HT (60 nM; 12 Ci mmol^{-1}, Radiochemical Centre, Amersham) for 10 min in the presence of 10 μM pargyline, 0·1 μM desmethylimipramine and 1 μM benzotropine. Krebs-bicarbonate containing 0·02% EDTA and ascorbic acid was used in all uptake and release studies. Slices were collected by rapid vacuum filtration on paper circles (Whatman No. 1) and for uptake studies the radioactivity was measured by scintillation spectrometry, or for release studies the paper filters were supported in Gelman filter chambers, superfused with medium (0·6 ml min^{-1}) and 4 min serial fractions collected (Beart & McDonald 1980). Statistical analyses were carried out on raw data using Student's *t*-test.

Slices of VTA avidly accumulated [^{3}H]-5-HT and a tissue:medium ratio of 18 ± 2 (4) was found after a 10 min incubation at 37 °C relative to 0 °C blanks. In further studies, initial velocities of uptake were determined over a range of 5-HT concentrations (0·03–45 μM). An Eadie-Hofstee plot of the initial velocity data was indicative of 5-HT uptake mediated by more than one component (Hutchinson & Haber 1975), and the data was partitioned before analysis employing a non-linear sigmoidal model (Vaughn et al 1976). These kinetic analyses indicated that two components of 5-HT uptake existed, with the K_m and V_{max} values being respectively 0·4 μM and 35 nmol g^{-1} h^{-1}, and 42 μM and 650 nmol g^{-1} h^{-1}. When the release of [^{3}H]-5-HT was examined, the efflux of the radiolabelled transmitter rapidly reached a steady baseline representing 3·3 ± 0·3% (mean ± s.e.m., n = 17) of the tissue stores per fraction, and a stimulus-associated release of [^{3}H]-5-HT was produced upon an 8 min exposure of the slices of VTA to either elevated potassium (44 mM) or protoveratrine A (100 μM; data not shown; Beart & McDonald 1980). The peak increases in the fractional release of [^{3}H]-5-HT were for potassium and protoveratrine A respectively, 73 ± 13% (n = 10) and 153 ± 25% (n = 7) of basal efflux. When Ca^{2+} was omitted during the period of potassium application the stimulus evoked release of [^{3}H]-5-HT was reduced by 72 ± 3% (mean ± s.e.m., n = 3; $P < 0·01$) of control. Tetrodotoxin (10 μM) completely blocked the protoveratrine A induced release of [^{3}H]-5-HT. The high-affinity uptake of [^{3}H]-5-HT by slices of VTA and its subsequent release by depolarizing stimuli provide evidence for the involvement of this transmitter in synaptic transmission (Fagg & Lane 1979), and suggest the presence of 5-hydroxytryptaminergic nerve terminals in VTA. Radioligand binding studies with [^{3}H]-5-HT (Bennett & Snyder 1976) provided preliminary evidence for 5-HT receptors in the VTA. Specific [^{3}H]-5-HT binding (14 nM [^{3}H]-5-HT) was 8 pmol g^{-1} and represented 50% of total binding, and 117% of the specific binding observed in a crude membrane preparation of the corpus striatum in parallel assays (Beart unpublished).

A possible interaction between 5-HT and DA was explored in a series of release experiments using the procedure described above. Slices of the VTA were loaded with [^{3}H]DA (10 μM; 6·7 Ci mmol^{-1}, Radiochemical Centre, Amersham) over a 10 min incubation in the presence of desmethylimipramine and chlorimipramine (both 0·1 μM), which were also present in the release media, and release experiments were performed as described previously (Beart & McDonald 1980). The spontaneous efflux of [^{3}H]DA rapidly reached a steady state level, with the amount released per fraction representing 4·3 ± 0·4% (mean ± s.e.m., n = 17) of the total tissues stores. An 8 min exposure of the slices to 5-HT (0·1–2 mM) produced small, reproducible

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Table 1. [^{3}H]Dopamine release by 5-HT. All values are mean ± s.e.m. of the number of experiments indicated. *$P < 0.05$ relative to control. Release studies were carried out as described in the text. Slices or homogenates of the VTA were loaded with [^{3}H]DA (10 μM) over a 10 min incubation. Desmethylimipramine and chlorimipramine (both 0·1 μM) were present to prevent non-specific uptake. The preparation employed for homogenate studies was an S_1 supernatant. Slices or homogenates were exposed to stimuli for 8 min—additional 4 min pre-exposure with methysergide. [^{3}H]DA release is given as the percentage of total tissue stores released and is the difference between the [^{3}H]DA present in a basal efflux fraction and that in the peak release fraction (defined as that containing the maximal amount of [^{3}H]DA).

Preparation	Stimulus	[^{3}H]DA release (% tissue stores)
Slices	100 μM 5-HT	1·2 ± 0·1 (17)
Slices	100 μM 5-HT + 25 μM methysergide	0·8 ± 0·1 (4)*
Slices	500 μM 5-HT	1·5 ± 0·2 (12)
Slices	2 mM 5-HT	1·8 ± 0·3 (4)
Homogenates	100 μM 5-HT	1·4 ± 0·5 (8)

increases in the efflux of [^{3}H]DA (see Table 1). Methysergide was able to attenuate this action of 5-HT (Table 1), and similar, but less reproducible effects were seen with 25 μM cyproheptadine (data not shown). This facilitatory effect of 5-HT upon [^{3}H]DA release could also be demonstrated in the presence of depolarizing stimuli (44 mM potassium and 100 μM protoveratrine A; see above). An 8 min exposure to protoveratrine A produced an increase in the efflux of [^{3}H]DA:peak release equivalent to 1·7 ± 0·4% (mean ± s.e.m., n = 3) of total tissue stores (spontaneous efflux subtracted). The stimulus-evoked release of [^{3}H]DA was potentiated by 132 ± 5% (mean ± s.e.m., n = 3; $P < 0.001$) of control in the presence of 2 mM 5-HT, and the peak release was 4·0 ± 0·2% (mean ± s.e.m., n = 3) of total tissue stores (spontaneous efflux subtracted). Similar effects were observed upon the potassium-evoked release of [^{3}H]DA, although the increase above potassium alone was only 61 ± 8% (mean ± s.e.m., n = 4; $P < 0.02$), but attenuated by 25 μM methysergide ($P < 0.05$). Quipazine (500 μM) was able to mimick the effect of 5-HT in that it potentiated the spontaneous and potassium-evoked efflux of [^{3}H]DA. In further studies, where slices were replaced by an homogenate (S_1 supernatant, Beart 1976) of the VTA, 5-HT was still able to release [^{3}H]DA (Table 1). Since neuronal connections should be completely severed in an homogenate, 5-hydroxytryptaminergic receptors may be localized on dopaminergic elements within the VTA. This result when considered with the evidence from the preceding release experiments suggests that 5-HT and its receptors may directly regulate DA release in the VTA.

The interaction between the 5-HT and dopamine systems within the VTA in vivo was studied by monitoring alterations in the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC), a biochemical index of dopaminergic nerve activity (Roth et al 1976), after the administration of drugs affecting 5-HT neurotransmission. The following drugs were injected intraperitoneally (2 ml kg^{-1}), quipazine (30 mg kg^{-1}, 60 min), 5-methoxy-*NN*-dimethyltryptamine (10 mg kg^{-1}, 30 min), metergoline (10 mg kg^{-1}, 90 min), chlorimipramine (25 mg kg^{-1}, 60 min, 5-hydroxytryptophan (100 mg kg^{-1}, 30 min) and *p*-chlorophenylalanine (2 × 300 mg kg^{-1}, 24 and 48 h). The VTA was homogenized in 5 volumes of ice-cold 0·1 M hydrochloric acid, 0·1% EDTA and stored overnight at −20 °C. Homogenates were centrifuged at 1000 *g* for 5 min at 4 °C, and portions of the supernatants were assayed for DOPAC by a radioenzymatic assay employing catechol-*O*-methyltransferase and [^{3}H]-*S*-adenosyl methionine (15–33 Ci mmol^{-1}, Radiochemical Centre, Amersham) in which DOPAC was estimated after conversion to [^{3}H]homovanillic acid (Beart & Gundlach 1980). [^{3}H]Homovanillic acid was isolated on paper chromatograms (Whatman No. 1, 46 × 4 cm), which were developed overnight with butan-1-ol–acetic acid–water (4:1:1 by volume). The spots corresponding to authentic homovanillic acid (R_F 0·83) were eluted with 2 ml of 2-methoxyethanol and after determination of the radioactivity present as [^{3}H]homovanillic acid, the DOPAC was quantitated using external and internal standards. The sensitivity of the assay, defined as the amount of DOPAC which, after conversion to [^{3}H]-homovanillic acid, gave twice the radioactivity present in the reagent blank, was 116 ± 32 pg (mean ± s.e.m., n = 6).

Table 2. 5-HT-like drugs and DOPAC in the ventral tegmental area. All values are the mean ± s.e.m. of the number of animals indicated in parentheses. Rats received the appropriate drug or vehicle intraperitoneally. DOPAC concentrations were estimated as described in the text. Control DOPAC concentration in the VTA was 325 ± 30 (14) ng g^{-1} wet wt.

Drug	Dose, time (mg kg^{-1})	DOPAC concentration (% control)
5-Methoxy-*NN*-dimethyltryptamine	10, 30 min	314 ± 69 (4)**
Quipazine	30, 60 min	52 ± 5 (4)†
Metergoline	10, 90 min	226 ± 21 (3)†
Chlorimipramine	25, 60 min	122 ± 13 (4)
5-Hydroxytryptophan	100, 30 min	77 ± 22 (4)
p-Chlorophenylalanine	2 × 300, 24 and 48 h	74 ± 5 (4)††

** $P < 0.025$, † $P < 0.02$, †† $P < 0.01$, ‡ $P < 0.001$ relative to control.

All of the 5-HT-like drugs altered the concentration of DOPAC in the VTA, and these data thus provide in vivo evidence for a 5-hydroxytryptaminergic modulation of dopamine release within this area. Although systemically administered drugs are unlikely to act selectively upon a single population of neurons, the preceding release experiments have indicated that 5-HT

receptors directly regulate DA release and the drug-induced alterations of DOPAC can therefore be interpreted in the light of the demonstration of this direct interaction. Thus the opposite effects of metergoline and quipazine on the concentration of DOPAC are consistent with the ability of these two drugs to block and accentuate the inhibitory action of 5-HT respectively (Fuxe et al 1978; Hamon et al 1976), and thereby increase and decrease the release of DA in the VTA. Their actions provide further evidence for a direct modulation of dopaminergic transmission by 5-HT. Although 5-methoxy-*NN*-dimethyltryptamine possesses activity as a 5-HT agonist, the drug has different actions to quipazine (Green et al 1981; Hamon et al 1976), and the large increase in the concentration of DOPAC may result from the ability of this indoleamine to activate presynaptic 5-HT receptors (autoreceptors; Hamon et al 1976) and hence disinhibit the postsynaptic dopaminergic neurons. Our observations in the VTA do not take account of 5-HT-DA interactions in the nucelus accumbens septi (Costall & Naylor 1978; Pycock et al 1978; Waldmeier 1980), which contains axonal terminals of A10 (mesolimbic) dopaminergic neurons, and where similar alterations in the concentration of DOPAC were also observed (Beart & McDonald, unpublished), but this interaction will not influence DA release in the VTA.

The present neurochemical findings, when considered with anatomical evidence of a 5-hydroxytryptaminergic innervation (see above) and with the high concentration of 5-HT within the VTA (Saavedra et al 1974), strongly suggest the presence of 5-hydroxytryptaminergic nerve terminals within this brain area, 5-HT receptors also appear to exist within the VTA and they can modulate DA release both in vitro and in vivo. Since DA release within the VTA is likely to be associated with dendrodendritic and dendroaxonic mechanisms within the somatodendritic area of A10 dopaminergic neurons (Beart & Gundlach 1980; Beart & McDonald 1980; Yim & Mogenson 1980), the situation in the VTA seems to be similar to the adjoining substantia nigra where 5-HT receptors are localized on the dendrites of A9 dopaminergic neurons (Reubi et al 1978) and can functionally modulate their activity (Giambalvo & Snodgrass 1978). Thus 5-hydroxytryptaminergic nerve terminals are in close apposition with DA release sites within the VTA, and are likely to play an important role in the regulation of the activity of the somatodendritic regions of A10 dopaminergic neurons (c.f. Herve et al 1979). Our data do not reveal whether this interaction also reduces the activity in ascending dopaminergic axons or whether the modulation of neuronal activity is restricted to the somatodendritic area. 5-HT clearly plays an important role in the regulation of the activity of mesolimbic dopaminergic neurons both in somatodendritic and nerve terminal regions.

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