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The involvement of 5-hydroxytryptamine in the release of dendritic dopamine from slices of rat substantia nigra

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[^{3}H]dopamine (DA) accumulated by slices of rat substantia nigra was released following superfusion with Krebs solution containing 5-hydroxytryptamine (5-HT) in a dose-dependent fashion with maximal release being produced by 1×10^{-4} M. This 5-HT-induced release of [^{3}H]DA was calcium-dependent and was inhibited by pretreatment with the 5-HT antagonist cinanserin. These results are interpreted as supporting the possibility that 5-HT has a role in regulating dopaminergic activity in the nigro-striatal pathway.

The afferent 5-hydroxytryptaminergic innervation to the substantia nigra originates from the medial and dorsal raphe nuclei (Bobillier et al 1976; Fibiger & Miller 1977; Dray et al 1978) and terminates predominantly in the zona reticulata of the nigra (Palkovits et al 1974). The potassium-induced release of 5-hydroxytryptamine (5-HT) from nigral slices in-vitro confirms the presence of nigral 5-hydroxytryptaminergic terminals (Reubi & Emson 1978). Both the electrophoretic application of 5-HT to cells in the substantia nigra (Aghajanian & Bunney 1975) and the electrical stimulation of the raphe nuclei (Davies & Tongroach 1978), result predominantly in inhibition in the nigra.

It has been suggested that the descending striato-nigral GABA-ergic pathway regulates neuronal activity in the ascending, dopaminergic, nigro-striatal pathway (Ribak et al 1976). Furthermore, this regulation may be modulated by a 'local circuit' in the nigra consisting of dendritic release of dopamine leading to enhanced release of γ-aminobutyric acid (GABA) (Reubi et al 1977) which in turn inhibits the dopaminergic nigro-striatal pathway. Within the substantia nigra dopamine has been shown to be contained in dendrites (Bjorklund & Lindval 1975) and to be released from them (Geffen et al 1976). The dopamine thus released has the potential to activate either autoreceptors, thereby regulating its own release (Groves et al 1975) or dopaminergic receptors located on the terminals of the GABA-ergic striato-nigral afferents. Further evidence supporting a role for dendritic release of dopamine has been the demonstration of a dopamine-sensitive adenylate cyclase located on terminals of afferents to the nigra (Premont et al 1976; Gale et al 1977; Minneman & Cuello 1979).

Hery et al (1980) showed that the unilateral nigral application of dopamine reduced the release of endogenously synthesized 5-HT from both the ipsilateral striatum and substantia nigra, suggesting that the activity of the raphe-nigral neurons may be regulated by dopamine released from dendrites of the nigro-striatal dopaminergic pathway. We have recently shown (Davies & Williams 1983) that 5-HT has a marked effect on the ability of GABA-ergic mechanisms to potentiate the cataleptogenic effects of the dopaminergic antagonist α-flupenthixol. The possibility that this effect was mediated in the substantia nigra has now been investigated in this present study where we have examined the action of 5-HT on the release of nigral dendritic dopamine in-vitro.

METHODS

Female Wistar rats, 150 ± 20 g, were decapitated and the brain rapidly removed. A transverse cut at the level of the posterior colliculus and a further cut 3 mm rostral to the anterior tip of the pineal body allowed the cerebellum to be removed and the substantia nigra to be dissected out. The white peduncular layer was removed and the nigra was immediately immersed in ice-cold Krebs solution

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and 300 μm slices prepared with a McIlwain tissue chopper. The slices were preincubated in Krebs at 37 °C continuously oxygenated with a 95% O_2/5% CO_2 mixture for 15 min after which the Krebs medium was replaced with Krebs containing [^{3}H]DA (specific activity 8·6 Ci mmol^{-1}) for 30 min. The tissue slices were then transferred to a superfusion chamber and the chamber immersed in a water bath at 37 °C. The slices were superfused with oxygenated Krebs at 37 °C at a flow rate of 0·5 ml min^{-1} and the 1 ml aliquots of superfusant were collected every 2 min for scintillation counting. To each sample of superfusant, 10 ml of scintillation cocktail was added and the activity counted on a Philips P.N. 4540 analyser. On completion of the superfusion the tissue slices were removed and solubilized by adding 1 ml of Protosol and incubated at 50 °C overnight. The tissue activity was again measured by adding 10 ml of the appropriate scintillation cocktail.

The effect of 5-HT on the release of [^{3}H]DA

Slices, prepared as described above, were incubated with [^{3}H]DA 1 × 10^{-6} M for 30 min. Previous studies had revealed that the tissue uptake of [^{3}H]DA reaches a plateau between 30–60 min yielding a tissue/medium ratio of approximately 80:1 and that superfusion of the tissue preparation with Krebs for 60 min resulted in a steady basal release being achieved. To ensure this the tissue was superfused for 70 min with normal Krebs solution. Between 70–72 min the tissue was superfused with Krebs containing a range of 5-HT concentrations (1 × 10^{-4}–1 × 10^{-8} M) from a separate reservoir. From 72–90 min superfusion with normal Krebs was reinstated. The activity in each 2 min aliquot of superfusant was measured and expressed as a percentage of the mean basal release observed in the three 2-min intervals before the superfusion with 5-HT (Cheramy et al 1978).

The effect of cinanserin on 5-HT-induced release of DA

These experiments were performed essentially as above with the exception that the tissue superfusion between 66–70 min was with Krebs containing cinanserin hydrochloride (1 × 10^{-5}–1 × 10^{-8} M), superfusion between 70–72 min was with Krebs containing 5-HT (1 × 10^{-5} M) drawn from a separate reservoir and from 72–90 min superfusion with normal Krebs was re-established. An assessment of the effect of cinanserin alone on DA release was evaluated by superfusing the tissue preparation with Krebs containing cinanserin (1 × 10^{-5} M) between 66–70 min and comparing the subsequent release of DA to that produced by superfusion with normal Krebs.

The effect of Ca^{2+} depletion on the 5-HT-induced release of DA

Tissue superfusion was in the usual manner except that the Krebs solution used for the superfusion was devoid of Ca^{2+} but of the same osmolarity as normal Krebs. It also contained 1 × 10^{-4} M ethylene glycol tetracetic acid (EGTA) to chelate endogenous tissue Ca^{2+}. The amount of transmitter released by 5-HT (1 × 10^{-5} M) between 70–90 min following the 2 min superfusion with 5-HT was evaluated and expressed as a percentage of the pre-stimulation release. This value was compared to that released by 5-HT in normal Krebs.

MATERIALS

Cinanserin hydrochloride was a gift from Squibb Europe Inc; [^{3}H]dopamine was supplied by Amersham International and 5-HT by Sigma Chemical Co.

Krebs—Bicarbonate medium; contained (g litre^{-1}): NaCl, 6·92; KCl, 0·354; $CaCl_2.2H_2O$, 0·280; $MgSO_4.7H_2O$, 0·144; KH_2PO_4, 0·162; $NaHCO_3$, 2·10; glucose, 2·00.

Scintillation cocktails. (i) For tissue samples: 5 g 2·5-diphenyloxazole; 0·5 g 1,4-d-i-2-(5-phenyloxazole)-benzene was dissolved in 1 litre of scintillation grade toluene and stirred mechanically for 4 h. (ii) For superfusant samples: As above with 500 ml of Triton X-100 added per litre of scintillation cocktail.

RESULTS

The effect of 5-HT on the release of [^{3}H]DA

[^{3}H]DA was released in a dose-dependent fashion with maximal release being produced by 5-HT, 1 × 10^{-4} M (Fig. 1). The perfusion with 5-HT (1 × 10^{-5} M) resulted in a significant and long-lasting release of [^{3}H]DA, which was significantly greater than that produced in control preparations at all times measured in the 8 min following superfusion, maximal release being achieved between 4–6 min (Fig. 3).

The effect of cinanserin on the 5-HT-induced release of [^{3}H]DA

Pretreatment with cinanserin 1 × 10^{-8} to 1 × 10^{-5} M inhibited the 5-HT-induced release of [^{3}H]DA in a

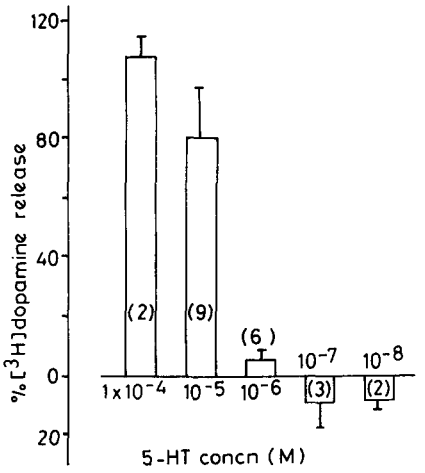

FIG. 1. Dose response effect of 5-HT on the release of [^{3}H]dopamine. Values given represent the mean (± s.e.m.) of the 5 (2 min) samples between 70–80 min expressed as a percentage of the mean of the 3 (2 min) prestimulation values, i.e. between 64–70 min. Numbers in parentheses indicate the number of experiments.

dose-dependent manner with maximal inhibition being produced by 1×10^{-5} M (Fig. 2).

A comparison of the effect of cinanserin (1×10^{-5} M) pretreatment on the 5-HT-induced release of [^{3}H]DA is given in Fig. 3. Pretreatment with cinanserin (1×10^{-5} M) did not significantly affect the basal release of [^{3}H]DA.

The effect of Ca^{2+} depletion on 5-HT-induced release of [^{3}H]DA

The removal of Ca^{2+} from the Krebs solution resulted in an 80% inhibition of the 5-HT-induced (1×10^{-5} M) release of [^{3}H]DA.

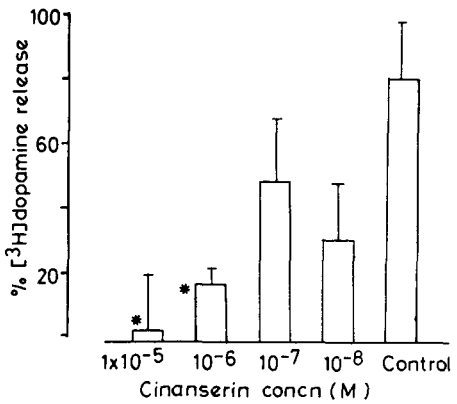

FIG. 2. Dose response effect of cinanserin on the 5-HT-induced (1×10^{-5} M) release of dopamine. Calculations as for Fig. 1. *$P < 0.001$ Statistical analysis by Student's *t*-test.

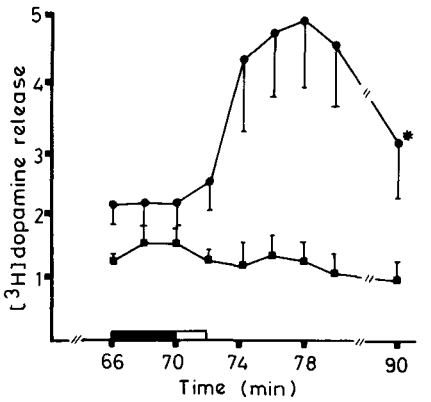

FIG. 3. The effect of cinanserin (1×10^{-5} M) on the 5-HT-induced (1×10^{-5} M) release of [^{3}H]dopamine. Values given represent the amount released (± s.e.m.) in each 2 min fraction expressed as a percentage of total tissue content. ■——■ Cinanserin + 5-HT (n = 9); ●——●; Krebs + 5-HT (n = 9); ▬ Cinanserin; ▭ 5-HT *$P <$ 0·001 results were analysed by a non-parametric multivariate test (Mantel & Valand 1970).

DISCUSSION

Although 5-HT has been localized in nerve terminals of the pars reticulata of the substantia nigra (Pickel et al 1975), and the activity of tryptophan hydroxylase in this region is as high as in any other part of the central nervous system (Brownstein et al 1975), its exact function is far from certain.

Geffen et al (1976) demonstrated a potassium-evoked release of dopamine from dendrites in the substantia nigra which was calcium-dependent. The present demonstration that 5-HT also released DA from slices of rat substantia nigra in both a Ca^{2+} and dose-dependent fashion suggests that the raphe-nigral 5-hydroxytryptaminergic pathway may serve an important function in the regulation of nigro-striatal dopaminergic activity. How this 5-HT-induced release of nigral DA is achieved is debatable, although a similar release of newly synthesized DA from labelled tyrosine by 5-HT in isolated striata has been reported (Besson et al 1969). However, the 5-HT-induced release of DA from nigral dendrites may be attributed to 5-HT activation of autoreceptors resulting in the removal of the normal inhibition which 5-HT exerts upon dendritic DA release. An alternative possibility is that 5-HT may release dendritic dopamine by activating postsynaptic 5-hydroxytryptaminergic receptors located on the dendrites. The ability of cinanserin to inhibit the 5-HT-induced release of dopamine in a dose-dependent fashion suggests that cinanserin may either selectively block 5-HT autoreceptors, producing an increased release of 5-HT with an associated

reduction in dendritic DA release, or be acting post-synaptically. The failure of cinanserin applied microiontophoretically to the raphe to block the effects of 5-HT argues against an autoreceptor effect (Haigler & Aghajanian 1977). Thus, it is probable that 5-HT is acting on post-synaptic receptors located on dopaminergic dendrites.

DA has been shown to release GABA from slices of rat substantia nigra (Reubi et al 1977), and a DA-sensitive adenylate cyclase has been demonstrated on terminals of striato-nigral neurons (Premont et al 1976; Gale et al 1977; Minneman & Cuello 1979). However, more recent studies (Hery et al 1980) suggest that DA receptors may also be located on the terminals of efferent 5-hydroxytryptaminergic systems in that unilateral intra-nigral application of DA reduced 5-HT release from both ipsilateral substantia nigra and caudate nucleus. This may well be a local feedback mechanism that regulates the described 5-HT-induced release of DA.

If 5-HT does bring about a release of GABA via dopamine then the intra-nigral administration of 5-HT or 5-HT antagonists should be expected to reduce and increase respectively DA-ergic activity within the ipsilateral caudate nucleus. Indeed, the intranigral administration of 5-HT or the 5-HT uptake inhibitor Wy 25093 (1-[1-([indol-3-yl]methyl)piperide-4-yl]-3-benzoylurea HCl) has been shown to produce ipsilateral circling and a fall in striatal DA turnover (James & Starr 1980), whereas the 5-HT antagonist, methysergide, produced contralateral turning. It has also been demonstrated (Tanner 1978) that unilateral nigral injection of the tryptophan-hydroxylase inhibitor, *p*-chlorophenylalanine, elevated striatal concentrations of the DA metabolite, homovanillic acid (HVA), suggesting an increased release of DA from nigro-striatal terminals. Conversely, intra-nigral 5-HT decreased striatal HVA whereas methysergide increased it (Straughan & James 1978). These data support the idea of 5-HT regulating nigro-striatal dopaminergic activity.

In summary, it is proposed that the 5-hydroxytryptaminergic regulation of nigro-striatal dopaminergic activity could be mediated via a post-synaptic action of 5-HT which releases dendritic dopamine and in turn leads to a release of GABA.

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