Vasopressin release by antagonists of GABA and glycine

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In cats anaesthetized with chloralose, nicotine releases vasopressin without oxytocin when acting from the ventral surface of the brain stem. To obtain this effect nicotine was injected into the cerebral ventricles or, more usually, applied topically through perspex rings (20 µl. into each ring of a solution of 2 or 4 mg/ml) to a bilateral region lateral to the pyramids and 6 to 9 mm caudal to the trapezoid bodies (Bisset, Feldberg, Guertzenstein & Rocha e Silva Jr, 1975). The vasopressin release occurred almost instantaneously and was short-lasting. It was followed by a paralyzing action rendering the region insensitive to renewed nicotine application. Hexamethonium similarly applied shared with nicotine the paralyzing action but did not itself stimulate vasopressin release (Bisset & Feldberg, unpublished experiments).

Using the procedure of Bisset *et al.*, there was no vasopressin release on topical application of physostigmine (10 to 50 mg/ml) carbachol (6 mg/ml), tetra-ethyl and tetra-methyl ammonium iodide (5–20 mg/ml) to the nicotine sensitive region, nor on injection into the cerebral ventricles of noradrenaline (100 µg) or morphine sulphate (750 µg), the morphine being injected into unanaesthetized cats. But the topical application of tubocurarine (20 mg/ml), for only a few minutes, released large amounts of vasopressin. In contrast to the effect of nicotine the release proceeded gradually and reached its maximum after about 1 hour.

On account of its neuromuscular and ganglion blocking action, tubocurarine might have been expected to act like hexamethonium. However, in the brain, tubocurarine produces strong long-lasting excitation thought to be due, at least partly, to dis-inhibition, i.e., antagonizing the action of GABA released from central inhibitory neurones. If this were the mechanism of its vasopressin releasing property other GABA antagonists, like picrotoxin, bicuculline and leptazol should have the same effect, and so they had. With regard to their other central actions, picrotoxin and bicuculline were found to be more, and leptazol less potent than tubocurarine; the leptazol effect was also of much shorter duration. The same differences seem to apply to their vasopressin releasing property. On topical application through the perspex rings, picrotoxin and bicuculline were effective in concentrations of 2 mg/ml., leptazol of 50 mg/ml. With leptazol the release ceased shortly after its removal, but continued when left inside the rings. Strychnine, an inhibitor of glycine, also released vasopressin on application to the nicotine sensitive region in concentrations of 5–10 mg/ml.

It is suggested that vasopressin release in the body is continuously inhibited by inhibitory neurones in the medulla which release GABA and glycine.

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Reference

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Morphine selectively blocks dopamine-stimulated cyclic AMP formation in rat neostriatal slices

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Many of the varied effects of the opiate narcotics may be due to a primary interaction with cyclic nucleotide systems. In both rat brain and cultured cells, morphine markedly affects both cyclic AMP and cyclic GMP metabolism (Minneman & Iversen, 1976). Behavioural and neurochemical evidence suggests that morphine may antagonize the effects of dopamine *in vivo*, in a manner differing significantly from neuroleptic drugs (Kuschinsky & Hornykiewicz, 1972). We report here a potent inhibitory effect of morphine on dopamine-stimulated cyclic AMP formation in intact slices of rat neostriatum.

Slices (260 × 260 µm) were prepared from rat neostriata as described previously (Minneman & Iversen, 1976). Drugs and preincubated tissue were added to a final volume of 250 µl medium and incubated for 15 min with no added phosphodiesterase inhibitor. The reaction was stopped by boiling and the

Table 1 Effect of morphine on agonist-stimulated cAMP production in rat neostriatal slices

	cAMP (pmol/mg protein)	*% Basal level*
Basal	3.08 ± 0.07	100
1 μM Morphine	2.18 ± 0.42	71
10 μM Isoprenaline	8.44 ± 0.30	274
10 μM Isoprenaline + 1 μM Morphine	8.50 ± 0.15	276
100 μM Adenosine	6.88 ± 0.61	223
100 μM Adenosine + 1 μM Morphine	7.12 ± 0.62	231
10 μM Prostaglandin E_1	9.26 ± 0.52	301
10 μM Prostaglandin E_1 × 1 μM Morphine	9.08 ± 0.31	295
100 μM Dopamine	6.32 ± 0.21	205
100 μM Dopamine × 1 μM Morphine	3.15 ± 0.12	102

cyclic AMP content of the supernatant determined as described previously.

In confirmation of the results of Forn, Krueger & Greengard, (1974) both dopamine and the β-adrenoceptor agonist isoprenaline caused increases in cyclic AMP formation in striatal slices (Table 1). Adenosine and prostaglandin E_1 (PGE_1) also both caused cyclic AMP increases (Table 1) and the latter two responses were not affected either by propranolol (10 μM) or by α-flupenthixol (1 μM), suggesting that there are several pharmacologically distinct receptors regulating cyclic AMP formation in rat neostriatum.

Morphine at concentrations from 10^{-7} to 10^{-4} M, caused a 30–50% decrease in cyclic AMP levels in striatal slices, however up to a concentration of 10^{-4} M, morphine did not affect basal adenylate cyclase activity in striatal homogenates.

Morphine (10^{-6} M) also caused a complete inhibition of the dopamine-stimulated cyclic AMP levels in striatal slices (Table 1). On the other hand, morphine up to a concentration of 10^{-3} M did not significantly affect the increases in cyclic AMP elicited by isoprenaline, adenosine, or PGE_1 (Table 1), suggesting that the opiate effect in the striatum is selective for dopamine. Morphine did not inhibit the dopamine-sensitive adenylate cyclase in striatal homogenates, except at very high concentrations (10^{-3}M). The effect of morphine, therefore, appears to require intact cells.

The effect of morphine on both basal and dopamine-stimulated cyclic AMP levels in striatal slices was blocked by naloxone (1 μM), suggesting that it is mediated through specific opiate receptors.

These results may provide a biochemical basis for the observed antagonistic effects of opiates on dopamine receptor-mediated behaviour *in vivo*.

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In vitro studies on the inhibition of monoamine uptake by Org 6582

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In vivo studies have revealed that Org 6582 (dl-8-chloro-11-antiamino-benzo-(b)-bicyclo[3.3.1] nona-3,6a (10a) diene hydrochloride) is a potent long acting inhibitor of rat brain 5-hydroxytryptamine (5-HT) uptake. In contrast to its effect on 5-HT uptake Org 6582 does not inhibit the *in vivo* central uptake of noradrenaline (NA) and dopamine (DA) (Goodlet, Mireylees & Sugrue, 1976; Sugrue, Goodlet & Mireylees, 1976). The objective of this study was to investigate the effects of Org 6582 on monoamine uptake *in vitro*. Drug effects on the uptake of [^{3}H]-NA and [^{3}H]-5-HT were studied using a crude synaptosomal fraction obtained from rat hypo-