Effects of morphine, levorphanol, nalorphine and naloxone on the release of acetylcholine from slices of rat cerebral cortex and hippocampus

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Morphine increases the total brain acetylcholine (ACh) concentrations in rats (Giarman & Pepeu, 1962) and mice (Hano, Kaneto, Kakunaga & Moribayashi, 1964) and depresses the release of ACh into the cerebral ventricles of the cat (Belesin & Polak, 1965). Thus it seemed of interest to study the effects of various narcotic analgesic drugs, as well as their antagonists, on the potassium stimulated release of ACh from slices of rat cerebral cortex and hippocampus, two areas of the brain with a high concentration of ACh.

Slices of either cortex or hippocampus were incubated for 60 min in a modified Krebs solution, containing 25 mM potassium, in the presence or absence of drug. The tissue incubates were stored at −20° C and subsequently assayed for ACh on the frog rectus abdominis muscle sensitized with neostigmine. The drugs were studied in the concentration range 0·03 mM–1·0 mM.

In cortical slices, 1 mM solutions of the narcotic agonists, morphine and levorphanol, and the narcotic antagonist, naloxone significantly decreased the potassium evoked release of ACh. In low doses (0·04 mM) levorphanol appeared to increase the release of ACh, but this increase was not statistically significant, whilst nalorphine, another narcotic antagonist, increased the release of ACh in all doses. In rat hippocampal slices, on the other hand, the release of ACh was stimulated by morphine but inhibited by nalorphine, levorphanol and naloxone at a 1·0 mM concentration.

The stimulant effect of morphine (1·0 mM) on the ACh release from hippocampal slices was antagonized by nalorphine (0·3 mM), but the depressant effect of levorphanol (1·0 mM) on release of ACh from hippocampal or cortical slices was not.

The potassium stimulated release of ACh from both cortical and hippocampal slices was shown to be calcium (Ca^{++}) dependent; if the calcium concentration was either increased or decreased from the normal concentration (2·5 mM), the ACh output was reduced. However, the inhibitory effect of levorphanol on ACh release from cortical slices was only affected by extreme changes in Ca^{++} concentration; in contrast, an increased Ca^{++} concentration enhanced the stimulant effect of morphine on ACh release from hippocampal slices, while a reduced Ca^{++} concentration antagonized the effect of morphine.

Thus the effects of morphine and levorphanol on rat brain slices differed in three respects. First, morphine depressed the release of ACh from cortical slices but stimulated the release of ACh from hippocampal slices, whereas levorphanol depressed release from both preparations. Second, the action of morphine was antagonized by nalorphine, whereas the action of levorphanol was not. Third, the effect of morphine on ACh release was Ca^{++}-sensitive but the effect of levorphanol on ACh release was not.

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Effect of septal lesions on acetylcholine output from the cerebral cortex in the cat

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Adult cats were immobilized by a transection made at the mid-pontine pretrigeminal level by means of a stereotaxically oriented spatula under halothane anaesthesia. The anaesthesia was discontinued and the cats resumed spontaneous respiration. Blood pressure was normal and the temperature was maintained at 37° C. Electrocoagulative lesions were stereotaxially placed in the septal region according to the coordinates of the cat atlas of Jasper & Ajmone Marsan (1954). At the end of the experiments the position and extent of the lesions were checked by histological examination. Cats in which the lesions were not correctly placed were considered sham operated animals.

Acetylcholine (ACh) output was determined by placing collecting cylinders filled with eserinized Ringers solution on the frontal cortex according to the method of Mitchell (1963). Every 10 min the solution was changed and bioassayed on the dorsal muscle of the leech.

The results are expressed in Table 1. For each cat the mean of at least three collecting periods before and after drug administration was used. Under resting conditions there was no difference in ACh output between controls and cats with septal lesion. However, the increase in ACh output caused by intravenous administration of (+)-amphetamine sulphate (Pepeu & Bartolini, 1968) is completely prevented by the septal lesions and that induced by local application of hyoscine hydrobromide (Bartolini & Pepeu, 1967) is significantly reduced.

These results emphasize the importance of the septum in the cholinergic pathways ascending to the cerebral cortex.

TABLE 1. *Effect of amphetamine and of hyoscine on ACh output from the cerebral cortex in casts with septal lesions*

Drug	ACh output ng/10 min per cm^2 ± S.E. Controls	Septal lesions	Sham operation
None	13·9±1·2 (8)	11·8±0·6 (10)	11·4±0·9 (7)
Amphetamine 2·5 mg/kg i.v.	28·4±3·8 (8)*	11·7±1·0 (6)	20·1±1·3 (4)‡
Hyoscine 1 μg/ml locally	40·4±2·4 (8)†	23·6±2·2 (3)†	33·6±7·2 (3)‡

Number of cats in brackets.
* Different from none with $P=0·01$.
† Different from controls with $P=0·01$.
‡ Different from none with $P=0·01$.

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