Actions and interactions of microiontophoretically applied morphine with transmitter substances on brain stem neurones

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Numerous attempts have been made to link the effects of morphine with its ability to interact with putative neurotransmitters in the brain (Clouet, 1971 ; Way & Shen, 1971 ; Weinstock, 1971).

We have used the microiontophoretic technique to investigate the effects of morphine and its interactions with acetylcholine (ACh), (−)-noradrenaline (NA) and 5-hydroxytryptamine (5-HT) when applied to spontaneously active single neurones in the brain stem. Experiments were performed on partially cerebellectomized rats, anaesthetized with urethane (1·2–1·8 g/kg).

Microiontophoretic applications of morphine (0·5–1·0% solutions at 10–30 nA) for periods of 0·5–10·5 min increased the firing rate of 33 and reduced that of 17 out of 76 neurones studied. Excitation by morphine was often very powerful ; relatively slow in its onset ; lasted throughout the period of application and began to decay immediately the drug was switched off. Reduction in neuronal firing by morphine was in general gradual and usually continued after the end of the application, so that recovery was often prolonged.

When several applications of morphine were made to the same neurone an increase in the latency of onset and a reduction in the magnitude of the excitatory response was often observed (10 out of 13 neurones). This phenomenon was never observed with neurones inhibited by morphine (6 neurones).

Prolonged application of morphine blocked excitation by ACh, NA or 5-HT, although occasionally potentiation of the excitatory effects of these compounds was observed. When excitation by one substance was blocked by morphine, the excitatory effect of another was often unaffected, suggesting that there may be some specificity in the antagonistic actions. On the other hand neither inhibition by ACh, NA or 5-HT, nor excitation by glutamate or DL-homocysteic acid was ever affected by morphine.

There appeared to be no correlation between the effects produced by morphine and those of either ACh, NA or 5-HT when these compounds were applied to the same neurone.

It appears that morphine has complex actions when applied iontophoretically to brain stem neurones. Both excitatory and inhibitory effects can be observed and the excitation may show acute tolerance after repeated application of morphine. The excitatory effects of ACh, NA or 5-HT can be antagonized and in some instances potentiated by morphine.

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Amino acid antagonists and the depression of cuneate neurones by γ-aminobutyric acid (GABA) and glycine

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Evaluation of amino acid antagonists requires that both iontophoretic potency and specificity are tested. Previous experiments on cerebral cortical neurones indicated that

bicuculline, picrotoxin and (+)-tubocurarine were all GABA antagonists (Hill, Simmonds & Straughan, 1971, 1972a, b) but it was not possible to examine their specificity, as glycine was an unsuitable control agonist in this area. Other workers have found that picrotoxin and bicuculline are capable of antagonizing GABA on neurones of the cuneate nucleus, without affecting the depressions produced on the same neurones by glycine (Galindo, 1969; Kelly & Renaud, 1971) and it therefore seemed a suitable area in which to examine the specificity of (+)-tubocurarine in comparison with the other two substances. In addition we studied the antagonism of glycine by strychnine, as a further test of specificity and potency (Curtis, Hösli & Johnston, 1968).

All experiments were performed on cats anaesthetized with N_2O/halothane in oxygen and prepared as described by Hill *et al.* (1973). A quantitative estimate of the iontophoretic potency of the antagonists used was obtained by the method of Hill & Simmonds (1973). All antagonists were used as 5 mM solutions in 150 mM NaCl.

A comparison of strychnine and one other antagonist was made on eighteen neurones, and strychnine was found to antagonize glycine on every occasion that it was tested. Diffusional release from a 5 mM solution of strychnine was sufficient to produce marked antagonism of glycine expelled with a current of 20–40 nA. Currents of GABA producing a depression with a similar time course to that produced by glycine were little affected by strychnine.

When the specificity of strychnine was expressed as a ratio; *i.e.* shift in glycine dose response line divided by shift in GABA dose response line, a value of 36:1 was obtained. Examination of the other substances in a similar manner produced less clear cut results. Bicuculline and picrotoxin would only produce measurable dose response curve shifts when expelled with currents in excess of 50 nA, and shifts were generally small, even though iontophoresis of the antagonist was continued for over 20 minutes. Examination of specificity revealed a glycine:GABA shift ratio of 0·6:1, indicating a significant antagonism of glycine as well as GABA, with both picrotoxin and bicuculline. (+)-Tubocurarine was found to antagonize GABA with lower currents than those used to expel picrotoxin and bicuculline, possibly due to its charge structure allowing a more efficient expulsion from the micropipette. The glycine:GABA shift ratio, 0·9:1, revealed that (+)-tubocurarine was non-specific. With all three substances individual examples of a specific GABA antagonism could be obtained, but the overall specificity was low, as indicated by the above ratios.

(+)-Tubocurarine was about four times as potent as picrotoxin or bicuculline as a GABA antagonist, when compared current for current, although this means little in terms of concentration at the receptor. As an antagonist of glycine on these neurones, strychnine was striking both for its potency and specificity. This comparative study suggests that the use of presently available GABA antagonists for identification of GABA operated synapses should be approached with caution.

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