of ^{3}H-GABA uptake against the concentration of GABA in the medium revealed two major components, corresponding to a high and low affinity uptake process with values for the apparent Km of 40 μM and 650 μM and for maximum velocity of $6{\cdot}7\times10^{-8}$ and $6{\cdot}1\times10^{-7}$ (mol/min)/g wet wt. tissue respectively. The same values were obtained in the presence of AOAA (10^{-5}M) indicating that at this concentration AOAA does not affect the initial rate of entry of GABA into the retina.

The maximum potentiation of ^{3}H-GABA accumulation achieved was 80% and this only occurred when GABA aminotransferase (GABA-T) activity was inhibited by 100%. The lowest concentration of AOAA producing this effect on uptake was found to be 10^{-6}M ; below this concentration both the inhibition of GABA-T and the potentiation of ^{3}H-GABA accumulation became progressively smaller.

The inhibition of retinal GABA-T by AOAA was time-dependent and was not reversed by pyridoxal-5-phosphate (10^{-3}M) or by repeated washing of the tissue in fresh medium. AOAA also inhibited glutamic decarboxylase (GAD), but to a lesser extent than GABA-T, and the GAD activity was partially restored by pyridoxal-5-phosphate. The inhibition of GAD *in vitro*, but not *in vivo* (Baxter & Roberts, 1961), can explain why the endogenous levels of GABA and other amino acids were not found to be changed by AOAA *in vitro*. It seems unlikely therefore, that AOAA is able to increase the accumulation of radioactive GABA by the tissue by enhancing the amount of exchange diffusion with endogenous GABA pools. Although AOAA also significantly increased the retinal accumulation of radioactive L-aspartic acid ($P<0{\cdot}001$) presumably by inhibiting aspartate aminotransferase, it did not alter the accumulation of L-glutamic acid, L-glutamine, taurine, glycine, γ-aminoisobutyric acid or DL-dopamine.

The efflux of radioactivity from retinae loaded with ^{3}H-GABA was markedly reduced in the presence of AOAA at a concentration sufficient to inhibit GABA-T by 100%. Under these conditions the radioactivity released by control retinae is in the form of tritiated metabolites, whilst only GABA is released in the presence of AOAA (Goodchild & Neal, 1972). These findings suggest that AOAA potentiates the accumulation of ^{3}H-GABA by isolated retina by reducing the metabolism of the amino acid and hence reducing the efflux of radioactivity from the tissue in the form of radioactive metabolites.

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Increase in brain and spinal acetylcholine levels without antinociceptive actions following morphine administration in the frog

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The hypothesis that the analgesic effect of morphine and related drugs may involve a cholinergic mechanism has been repeatedly proposed and is supported by several observations. Among these, the rise in brain acetylcholine (ACh) following the administration of analgesic doses of morphine in rats (Maynert, 1967) and in mice (Harris, 1970) and the decrease in ACh output from the cerebral cortex in cats (Jhamandas, Phillis & Pinsky, 1971).

In the present study the effect of morphine on the ACh content of the brain and spinal cord was investigated in frogs (Rana esculenta). Morphine hydrochloride was administered S.C. in a volume of 0·2 ml frog saline and 20 min later the frogs were killed by decapitation. Brain and spinal cord removal and ACh extraction were carried out according to the procedure previously described (Nistri & Pepeu, 1972). The ACh content of the extracts was determined by bioassay on the dorsal muscle of the leech.

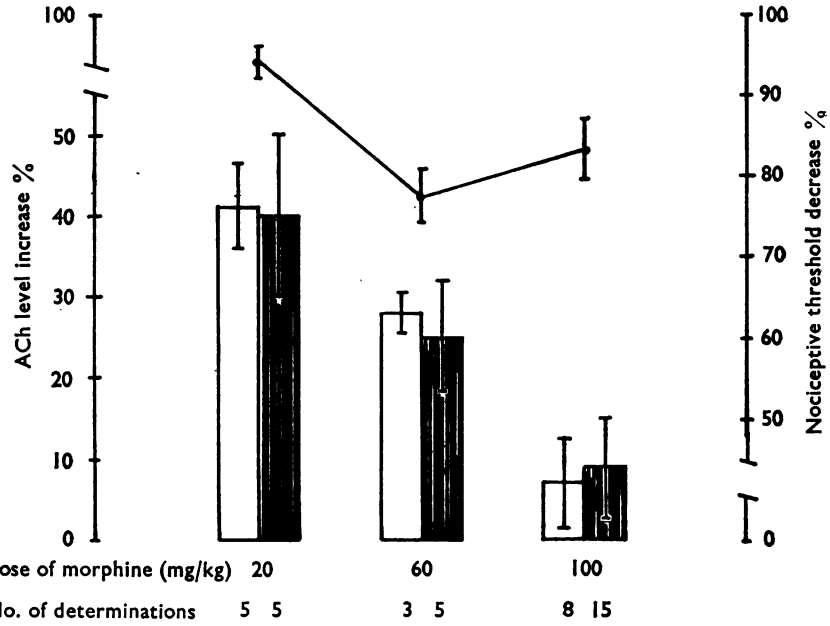

FIG. 1. Effect of morphine on brain and spinal ACh and on pain threshold. White columns: brain, hatched columns: spinal cord. In the untreated frogs the brain ACh was 4·28 ±0·34 and the spinal cord ACh was 2·40 ±0·13 μg/g ±S.E. (n=6), the pain threshold was 1·4 ±0·07 volts.

As shown in Fig. 1, the administration of morphine produced the largest increase in brain and spinal cord ACh at the smallest dose used. Using a large dose of morphine (100 mg/kg) an insignificant rise was observed. A close parallel between the effects in the brain and in the spinal cord was noted.

The antinociceptive effect was tested according to Leslie, Ireson & Tattersall (1969) by determining the jump threshold to a light electrical stimulation applied through an electrified grid at the bottom of the cage. As is also shown in Fig. 1, the threshold voltage was significantly reduced by morphine at the dose of 60 and 100 mg/kg. Confirmation of the lack of an antinociceptive effect was also provided by the unaltered response to a light mechanical nociceptive stimulation.

Therefore, if these methods are a reliable test for analgesia, doubt could be cast on the significance of the increase in brain ACh with regard to the antinociceptive action of morphine.

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