Effects of ethanol on acetylcholine and adenosine efflux from the in vivo rat cerebral cortex

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Ethanol has been reported to have analgesic properties (Bass et al 1978; Brick et al 1976) and James et al (1978) have suggested that the mode of analgesic action of ethanol might be similar to that of morphine. In this laboratory we have observed that ethanol, like morphine, depresses the release of acetylcholine (ACh) from the intact cerebral cortex (Phillis & Jhamandas 1971; Jhamandas et al 1971; Morgan & Phillis 1975). The effects of morphine are antagonized by methylxanthines, such as caffeine and theophylline (Jhamandas et al 1978). Methylxanthines also antagonize the reduction in ACh release elicited by the putative neuromodulator, adenosine (Jhamandas & Sawynok 1976), and since morphine enhances the release of adenosine from brain, the speculation has been offered that increased concentrations of extracellular purines may contribute to the effects of morphine (Phillis et al 1980a, b).

The possibility that ethanol, like morphine, may act by a purinergic mechanism is of considerable interest in the light of the persistent controversy regarding the use of coffee (caffeine) as an antidote for ethanol inebriation (Gilbert 1976; Stephenson 1977). Caffeine, a methylated xanthine in coffee, is a potent adenosine antagonist (Phillis et al 1979). If, in fact, ethanol action involves a purinergic link, then the use of coffee as an antidote for inebriation could be justified. In the light of these speculations we considered it appropriate to investigate the effects of ethanol on adenosine efflux from the rat brain.

Experiments were carried out on 20 male Sprague-Dawley or Wistar rats. Anaesthesia was induced with halothane and the trachea was cannulated. The animals were then placed in a stereotaxic head holder and anaesthesia was maintained with a mixture of nitrous oxide (75%); oxygen (25%) and methoxyflurane. After completion of the surgical procedures, the methoxyflurane vaporizer was adjusted to ensure that the animals would be lightly but adequately anaesthetized and no further alterations were made to the flow regulators. Body temperature was kept constant at 37 °C by a feedback circuit with a rectal probe. An intravenous cannula was placed in the right femoral vein.

Adenosine release. Both cortical hemispheres were exposed, leaving a thin crest of bone along the midline. The dura was removed and rectangular cups with inside dimensions of 5 × 8 mm were placed bilaterally on the cortical surface. Leakage from the cups was prevented by a thin coating of silicone grease on the cup surface in contact with the brain. When the cups were in place, the exposed cortical, bone and muscle surfaces around them were covered with a layer of 4% agar in 0·9% NaCl (saline). The cups were filled with a sterile, pyrogen free, saline (Ringer's Injection, Abbott). The solution in each cup was removed and replaced for 40 min with 100 μl [2,8-^{3}H]adenosine (0·1 mM; specific activity of 0·1 Ci mol $^{-1}$, ICN).

At the end of the incubation period, the cups were rinsed 10 times in rapid succession with warmed saline and subsequently refilled with 100 μl saline. Thereafter the cup contents were withdrawn every 15 min and replaced with fresh saline. The collected samples were mixed with 2 ml of PCS scintillation fluid and counted in a Nuclear Chicago Isocap 300 liquid scintillation counter.

Seven rats were injected intravenously with 0·25 g kg^{-1} ethanol 2½ h after the onset of collections with a subsequent injection of 1 g kg^{-1} 2 h later. Seven rats were given one dose (1 g kg^{-1}) of ethanol. Since the efflux of labelled material varied amongst animals, the tabulated results have been standardized by expressing post-drug release values as a percentage of the mean of efflux rates in the two collection periods immediately preceeding drug administration. The post drug efflux rates are also presented by pooling them into 30 min periods. The significance of drug effects on efflux was evaluated using Student's *t*-test.

Acetylcholine release. Six rats were used. Bone overlying the cerebral hemispheres was removed, including that in the midline and a single Lucite cup with an inside diameter of 1·1 cm was placed over both hemispheres. Exposed surfaces around the cup were covered with 4% agar in saline and the cup was filled with a solution of 5 × 10^{-5} gm ml^{-1} neostigmine bromide in saline. This solution was changed three times at 20 min intervals and the cup was then rinsed several times and filled with 0·6 ml of the neostigmine-containing solution. This solution was collected after 15 min and replaced with fresh solution. Subsequent collections were made at 15 min intervals. Ethanol (1 g kg^{-1}) was administered 1½ h after the start of the collections. The ACh content of each cortical perfusate was determined by bioassay on the hearts of *Mercenaria mercenaria*. At the end of each experiment the hearts were perfused with a solution of benzoquinonium chloride (5 × 10^{-7} M; Mytolon, an ACh antagonist)-containing sea water and in every instance the inhibitory effects of the cortical perfusates were abolished.

The release of adenosine and its metabolites from 27 cortices was studied. The amount of labelled material released showed an exponential decline which tended to

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reach a plateau phase 2–2¼ h after the end of the incubation period. Ethanol injections were administered 2½ h after the start of sample collecting.

The results of experiments on 13 cortices after administration of 0·25 g kg^{-1} ethanol and the results from 21 cortices after administration of 1 g kg^{-1} showed that ethanol caused a small, non-significant, rise in purine efflux from the cerebral cortex, and this lasted for only 30 min. The level of purine efflux then returned to control or sub-control values.

Ethanol (1 g kg^{-1}) was then tested for its effects on ACh release from 6 cerebral cortices. The mean control rate of release during the 30 min period preceeding ethanol administration was 49·13 ± 9·15 pg min^{-1} cm^{-2} (mean ± s.e.m.). Ethanol caused a significant ($0{\cdot}01 < P > 0{\cdot}025$) depression of ACh efflux during the 60 min following its administration. In the 60–90 min collection periods the rate of release started to recover and it had returned to control values 2 h after administration. The duration and amount of inhibition of ACh release observed in these experiments are similar to those previously observed in the cat cerebral cortex (Phillis & Jhamandas 1971; Morgan & Phillis 1975).

These results confirm previous reports that ethanol, like morphine, elicits a reduction in the rate of ACh release from the cerebral cortex. A reduction in ACh release from the brain would also be expected on the basis of findings that brain ACh concentrations are enhanced after administration of ethanol (Brown et al 1977). It has been suggested that many of the actions of ethanol on the central nervous system result from a decreased level of activity in the midbrain reticular formation (Kalant 1974) and this would be consistent with the present findings. ACh release from the cerebral cortex has been related to a diffusely spreading ascending cholinergic pathway associated with the reticular arousal system (Karczmar 1979). A reduction in the activity of this system would explain the sedative and electroencephalographic changes evoked by ethanol (Perrin et al 1974) and its action on cerebral ACh release.

Ethanol failed to elicit a significant increase in purine efflux from the cerebral cortex and in this respect ethanol differs in its actions from those of morphine (Phillis et al 1980). After the cerebral cortex has been prelabelled with [^{3}H]adenosine, the radioactive marker is associated with adenine nucleotides, inosine, adenosine, and hypoxanthine (Sulakhe & Phillis 1975; unpublished observations). There was an increase in the rate of purine efflux after ethanol administration but this was too small to be significant and lasted only for 30 min. An increase in extracellular adenosine concentrations is therefore unlikely to have been responsible for the reduction in ACh efflux, which lasted for up to 2 h. It is possible, however, that this small increase in extracellular adenosine would account for the stimulant effect of ethanol on cyclic AMP formation in rat cerebral cortical tissue (Volicer et al 1977).

In the light of our failure to observe a significant effect of ethanol, unlike morphine, on purine release from the cerebral cortex, it appears unlikely that ethanol exerts its central actions by a purinergic mechanism Our findings do not therefore provide evidence in support of the use of coffee (caffeine) as an antidote to ethanol intoxication.

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