Serotoninergic involvement in the effect of ethanol on body temperature in rats

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Among the many effects of ethanol, changes in body temperature are marked and readily measured. A hypothermic response occurs in a variety of species, including man. The mechanism(s) mediating the effects of ethanol on body temperature are unknown. Recently we have shown that ethanol affects both noradrenergic (Pohorecky, 1974) and serotoninergic neurons (Pohorecky, Jaffe & Berkeley, 1974) in the brain, and, since regulation of body temperature is believed to involve both noradrenergic and serotoninergic neurons, it became of interest to examine whether monoamines mediate the hypothermic effect of ethanol. Prostaglandins are also believed to be involved in the regulation of body temperature (Komiskey & Rudy, 1974). Recently it was reported that ethanol might interfere with prostaglandin synthesis (Collier, McDonald-Gibson & Saeed, 1975). We therefore tested the possibility that the hypothermic effect of ethanol was mediated through prostaglandins.

Male Sprague Dawley rats (Holtzman Laboratories), 200–250 g, were housed singly in a room maintained at a temperature of 21° with a 12:12 light/dark cycle. Food (Purina chow) and water were freely available. Ethanol was injected intraperitoneally as a 20% solution in sterile saline (Abbott Laboratories). Drugs were prepared in saline except for indomethacin, which was solubilized in propylene glycol, and probenecid, which was dissolved in a small amount of 0·1 N NaOH after which the pH and the volume were adjusted with 0·5 M phosphate buffer, pH 7·0. Generous gifts of drugs were provided by Smith Kline & French Laboratories (phenoxybenzamine), McNeil Laboratories (haloperidol), Merck, Sharp & Dohme Laboratories (indomethacin and probenecid), and Lilly Research Laboratories (Lilly 110140). α-Methyl tyrosine methyl ester was purchased from Sigma, and *p*-chloroamphetamine from Regis Chemicals. Rectal body temperature was measured with a YSI model 43 TA thermometer (Yellow Springs Instrument Co.), the rectal probe being lubricated with glycerol before insertion into the rectum (3 cm).

Groups of 12 rats were pretreated with saline, phenoxybenzamine (20 mg kg^{-1}), haloperidol (2 mg kg^{-1}), α-methyl tyrosine methyl ester (150 mg kg^{-1}), or indomethacin (4 mg kg^{-1}). Thirty min later (50 min later for indomethacin group), half of the rats in each group were injected with saline and the other half with ethanol (3 g kg^{-1}). Body temperature was recorded before treatments with the drugs and every 30 min thereafter for 2 h. Ethanol injection resulted in a significant drop of body temperature (−1·95°). Haloperidol by itself produced no significant change in body temperature, and pretreatment with it had no effect on the temperature drop caused by the injection of ethanol. Although phenoxybenzamine had a hypothermic effect of its own, pretreatment with it had no effect on the drop in temperature caused by the ethanol injection. Pretreatment of animals with α-methyl tyrosine, which blocks catecholamine synthesis, did not alter the hypothermic effect of ethanol.

To determine the possible mediation by prostaglandins of the hypothermic effect of ethanol, animals were pretreated with indomethacin, a drug known to inhibit prostaglandin synthesis (Vane, 1971). Indomethacin by itself did not affect body temperature, neither did it alter the hypothermic effect of ethanol.

We tested the possibility that ethanol alters body temperature through its known interaction with serotoninergic neurons (Pohorecky & others, 1974). *p*-Chloroamphetamine is a known inhibitor of central tryptophan hydroxylase which produces a very marked and prolonged depletion of brain 5-HT (Sanders-Bush, Bushing &

Sulser, 1972). Fig. 1A shows that *p*-chloroamphetamine by itself produced a slight rise in temperature; as expected, ethanol produced a fall, which was maximal at 90 min (−1·32°). However, the animals treated with both drugs showed a much more pronounced hypothermia (−2·4°) and the peak decline was reached sooner, at 60 min. This experiment showed that inhibition of 5-HT synthesis in the brain markedly potentiated the hypothermic effect of ethanol.

To test this hypothesis further, we carried out an experiment in which 5-HT reuptake was inhibited with 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenyl-propylamine hydrochloride (Lilly 110140), a drug known to specifically inhibit 5-HT uptake at presynaptic terminals, which presumably would therefore lead to increased stimulation of serotoninergic receptors (Fuller, Perry & Molloy, 1974). Results are shown in Fig. 1B. Treatment of rats with Lilly 110140 produced an increase in body temperature, which did not reach the limits of significance; it did, however, reduce the hypothermic effect of ethanol by 50% at 30 min after the injection of ethanol. The recovery of normal body temperature of the animals treated with both drugs was faster than that in those receiving just ethanol. Thus, at 60 min body temperature of those treated with both drugs was almost back to normal, whereas that of the group treated with ethanol alone was still low at 120 min.

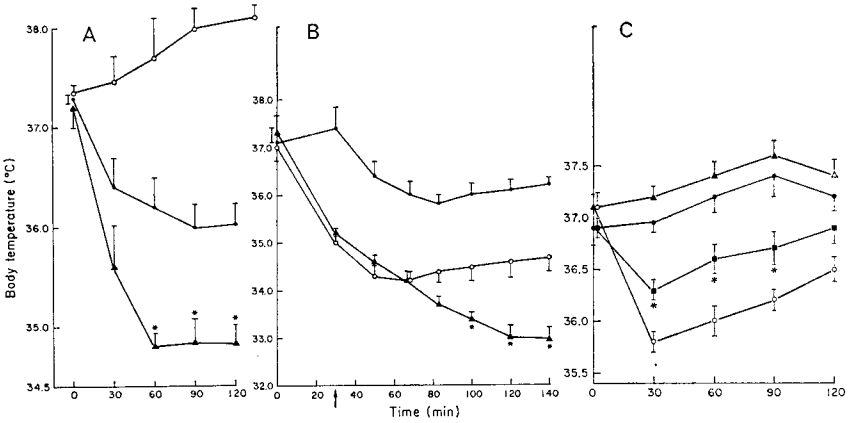

FIG. 1A. Potentiation of ethanol-induced hypothermia by *p*-chloroamphetamine. *p*-Chloroamphetamine (10 mg kg^{-1}, i.p.) was injected into 12 rats, and 6 rats were injected with saline. Twenty-four h later ethanol (3 g kg^{-1}) was injected into 6 of the rats pretreated with *p*-chloroamphetamine and into the 6 rats pretreated with saline; the other 6 rats pretreated with *p*-chloroamphetamine received a saline injection. Means ± standard error of the mean (vertical bars) are presented. ●—● Ethanol. ○—○ *p*-Chloroamphetamine. ▲—▲ *p*-Chloroamphetamine + ethanol.

B. Partial inhibition of the hypothermic effect of ethanol (2 g kg^{-1}) elicited by Lilly 110140 (10 mg kg^{-1} i.p., 2 h before ethanol). Data presented begin at the time when ethanol or saline were injected into animals pretreated with Lilly 110140 or saline. Each point is the mean of 5 animals and the vertical bars indicate ± standard error of the mean. ●—● Ethanol. ○—○ Probenecid. ▲—▲ Probenecid + ethanol.

C. Effect of probenecid on the hypothermic effect of ethanol. Probenecid (250 mg kg^{-1}) or saline was injected at zero time; ethanol (3 g kg^{-1}) or saline was injected 30 min later, as indicated by the arrow. Data are presented as means ± standard errors of the means of 5 animals. ●—● Saline. ▲—▲ Lilly 110140. ○—○ Ethanol. ■—■ Lilly 110140 + ethanol.

All the ethanol treated groups differed significantly (at least $P < 0{\cdot}005$) from their zero time control value. Asterisks indicate the means for the groups treated with drug + ethanol which differed ($P < 0{\cdot}05$ or more) from the corresponding groups treated with ethanol. Rectal temperature was recorded every 30 min (A, B) and every 20 min (C).

Finally, we pretreated animals with 250 mg kg^{-1} probenecid, a drug known to inhibit the transport of acid monoamine metabolites, including that of 5-HIAA, the direct metabolite of 5-HT, out of the brain (Neff, Tozer & Brodie, 1967). Fig. 1C shows that probenecid by itself caused a more marked 'hypothermia than did ethanol. The combination of the two drugs resulted in some prolongation and potentiation of the hypothermic effect.

The present experiments do not differentiate between the central and the possible peripheral effects of the various drugs used. Therefore, although these drugs have marked central effects, contribution by peripheral changes in monoamines cannot be excluded. Since the hypothermic response of ethanol was not modified in the present studies when catecholamine synthesis or their postsynaptic receptors were blocked, it appears that changes in body temperature produced by ethanol are not mediated by dopaminergic or noradrenergic mechanisms. Furthermore, the experiments using indomethacin to block the synthesis of prostaglandins suggest that the latter are not involved in ethanol hypothermia. However, when the synthesis of 5-HT was blocked with *p*-chloroamphetamine, ethanol-induced hypothermia was potentiated. That this effect was due to a depletion of 5-HT concentrations is suggested by the rather specific effect *p*-chloroamphetamine has on serotoninergic neurons (Sanders-Bush & Sulser, 1970) and the lack of other known interactions of ethanol with this drug. Furthermore, the partial inhibition of ethanol hypothermia by Lilly 110140, a specific inhibitor of 5-HT uptake, also supports the role of serotoninergic neurons in this effect of ethanol. The role of 5-HT in ethanol-induced hypothermia is supported by our observations that probenecid may potentiate this effect. This may be explained by an inhibition of 5-HIAA transport by probenecid, but other explanations are also possible.

These results suggest that ethanol may induce hypothermia by an effect on 5-HT metabolism at some site. Our experiments appear to indicate that the hypothermic effect of ethanol is brought about by reduced stimulation of receptors for 5-HT. These results do not preclude other explanations. For example, ethanol could change central concentrations of electrolytes (e.g., Ross, Medina & Cardenas, 1975) which may in turn alter body temperature (Myers, 1970).

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