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Ethanol-Induced Inhibition of the Drinking Response to Hypertonic Saline in the Rat

PHILIP HEYWARD[1] AND GRAEME EISENHOFER

Department of Physiology, Victoria University of Wellington, Wellington, New Zealand and Wellington Clinical School of Medicine, Wellington Hospital, Wellington 2, New Zealand

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HEYWARD, P. AND G. EISENHOFER. *Ethanol-induced inhibition of the drinking response to hypertonic saline in the rat.* PHARMACOL BIOCHEM BEHAV 22(3) 493–496, 1985.—The effects of ethanol, in doses of 0.05, 0.1 and 0.2 ml/100 g, on the drinking responses of rats to subcutaneous injection of hypertonic saline (1 mEq/100 g) were examined. Rats were also studied for the effects of ethanol (0.1 ml/100 g) on drinking responses to intraperitoneal injection of dextran (20% w/v, 1.5 ml/100 g). After injection of hypertonic saline, rats given ethanol drank less than those administered water or isocalorific glucose. Ethanol inhibited the drinking responses to hypertonic saline dose-dependently with higher doses having a greater inhibitory effect. Ethanol administration had no effect on water consumption stimulated by intraperitoneal injection of dextran. It is concluded that administration of ethanol to rats has a dose-dependent inhibitory effect on thirst and fluid consumption stimulated by injection of hypertonic saline but is without effect on thirst and drinking stimulated by intraperitoneal injection of dextran.

Thirst Dextran Water consumption Hypertonic saline Ethanol Drinking response

ETHANOL causes a state of negative water balance and dehydration through a transitory increase in free water clearance, this effect being secondary to a reduction in circulating plasma vasopressin concentrations [3]. Polydipsia after ethanol consumption [5,10] may be prevented by prior administration of vasopressin [2,4] indicating that thirst and increased fluid consumption after ethanol are secondary to dehydration. It has also been shown that ethanol consumption has inhibitory effects on the osmotic stimulation of thirst in man, although this effect is normally obscured by dehydration-induced thirst which becomes apparent as blood ethanol concentrations decline and release thirst centres from the inhibitory effects of ethanol [4]. We have extended these observations by examination of the effects of ethanol on drinking responses of rats to subcutaneous injection of hypertonic saline and intraperitoneal injection of dextran.

METHOD

Male rats of the Wistar strain weighing between 300 and 450 g were used. Rats were housed in pairs in a room maintained at 20°C and illuminated from 0700 to 1900 hr. Food (Wairarapa Stock Foods diet 86) and tap water were available ad lib but to ensure uniformity of ethanol absorption rats were deprived of food overnight prior to investigation. Studies were performed in a room maintained at 20°C, beginning between 1000 and 1100 hr. Subsequent to experimental manipulation, rats were placed in mesh-bottomed metabolism cages. Deionised water was made available from a drinking spout at the top of the cage connected by a closed syphoning system of flexible tubing to a reservoir. An isotonic force transducer (Harvard Apparatus) was used to monitor the mass of water contained in this reservoir, this measurement being displayed continuously on a chart recorder. Animals were habituated to the apparatus before experimentation. All solutions for injection were sterilised by millipore filtration. Differences between groups were assessed using the Wilcoxon test for independent samples. The significance of relationships was assessed using Spearman's rank correlation coefficient (r_s). Results are presented as the mean±S.E.

Hypertonic Saline Induced Drinking

In these studies drinking was induced by subcutaneous injection of hypertonic saline solution [6,16]. Rats received by stomach tube 0.05, 0.1 and 0.2 ml/100 g bodyweight doses of ethanol, as 5, 10 and 20% solutions respectively (n=6 per group). Sixteen control rats were given either deionised water (n=8), or glucose solution isocalorific with the 0.2 (n=4), 0.1 (n=2) and 0.05 (n=2) ml/100 g doses of ethanol. Twenty-five minutes later rats were subcutaneously injected with a local anaesthetic (lignocaine HCl) in the inguinal region and the injection site marked. After five minutes rats were injected subcutaneously at this site with an amount of

[1]Requests for reprints should be addressed to P. Heyward at present address: Department of Physiology, University of Melbourne, Parkville, Victoria 3052, Australia.

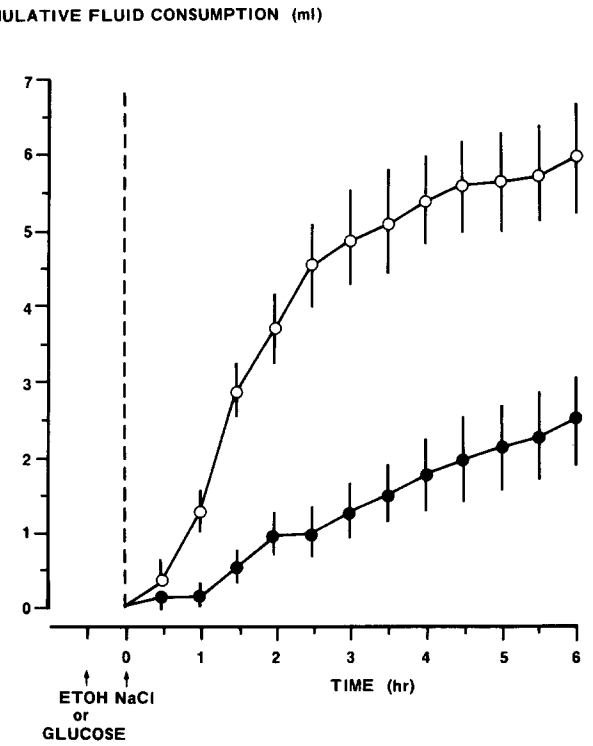

FIG. 1. Cumulative fluid consumption (mean±S.E.) for 6 hr after subcutaneous injection of hypertonic saline in rats administered ethanol (●, n=18) and isocalorific glucose or water (○, n=16). The various control groups, on one hand, and the three ethanol dosage groups, on the other, were pooled. Ethanol significantly ($p<0.005$) reduced the drinking response at all time intervals 2 hr and more after hypertonic saline.

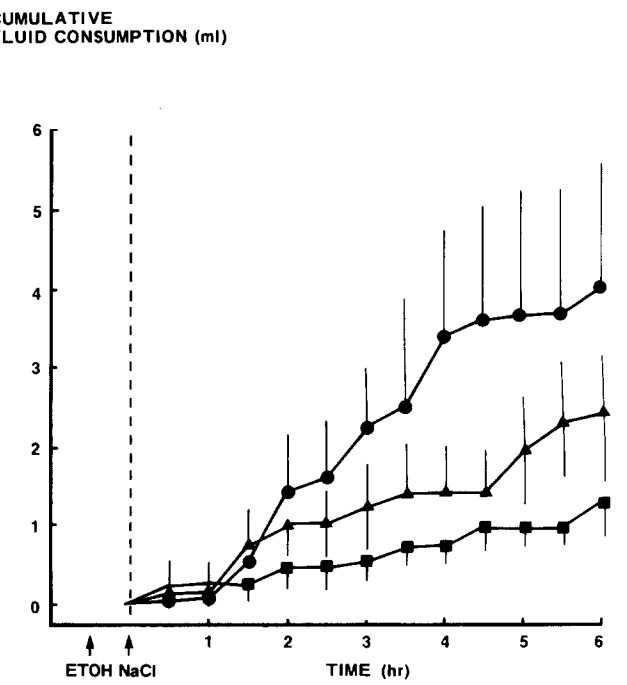

FIG. 2. Cumulative fluid consumption (mean±S.E.) for 6 hr after subcutaneous injection of hypertonic saline in rats administered 0.05 (●, n=6), 0.1 (▲, n=6) and 0.2 (■, n=6) ml/100 g ethanol. Significant ($p<0.05$) inverse correlations were found between increasing doses of ethanol and cumulative fluid consumption between 3.5 and 5.5 hr after hypertonic saline. Ethanol dose-dependently inhibited the drinking response to hypertonic saline.

15% sodium chloride solution equivalent to 1 mEq/100 g body weight and containing further anaesthetic. Investigations were also carried out, in which subcutaneous injection of isotonic (0.85%) saline was isovolumetrically substituted for the hypertonic saline, in two groups of rats given either 0.1 ml/100 g bodyweight doses of ethanol (n=6), or glucose isocalorific with this dose of ethanol (n=6). Drinking behaviour was observed for 6 hr after injection of saline.

Dextran-Induced Drinking

In these studies drinking was induced in rats by the intraperitoneal injection of hyperoncotic colloid [7, 8, 13]. Dextran [8] rather than polyethylene glycol [7,13] was chosen since preliminary studies indicated that it induced a much more reliable drinking response. Dextran (Sigma, MW 70,000–90,000) was injected intraperitoneally at body temperature as a 20% solution at the dose of 1.5 ml/100 g body weight, 90 min before administration of 0.1 ml/100 g ethanol (n=6) or isocalorific glucose (n=6). Animals were then placed in the apparatus and monitored until the end of 6 hr.

RESULTS

Rats receiving subcutaneous injection of isotonic saline, after 0.1 ml/100 g ethanol, drank 0.6±0.3 ml and those given glucose solution or water drank a total of 0.5±0.3 ml water 6 hr after isotonic saline injection. Drinking after hypertonic saline was significantly greater than that after isotonic saline injection in both ethanol treated rats ($p<0.05$) and control animals ($p<0.005$). No significant difference in water consumption existed between rats given glucose solution or deionised water under any of the experimental conditions.

For purposes of presentation the various control groups, on one hand, and the three ethanol dosage groups, on the other, were pooled. The control group consumed a mean (±S.E.) total of 6.1±0.7 ml water six hr after subcutaneous injection of hypertonic saline, while rats given ethanol showed a 6 hr cumulative water consumption of 2.5±0.6 ml, intake being significantly ($p<0.005$) reduced below that of control rats at all intervals 2 hr and more after hypertonic saline injection (Fig. 1).

The inhibition by ethanol of drinking induced by subcutaneous injection of hypertonic saline was found to be dose-dependent (Fig. 2). Rats given 0.05 ml/100 g ethanol consumed a total of 4.0±1.6 ml water over 6 hr, those given 0.1 ml/100 g drinking 2.4±0.7 ml and the 0.2 ml/100 g group drinking 1.3±0.4 ml. These differences were not significant, but significant ($p<0.05$) inverse relationships were found between increasing doses of ethanol (0.05, 0.1 and 0.2 ml/100 g) and cumulative fluid consumption at 4.0 ($r_s=0.49$), 4.5 ($r_s=0.47$), 5.0 ($r_s=0.50$) and 5.5 ($r_s=0.47$) hr after hypertonic saline.

Water consumption induced by intraperitoneal injection of dextran was not affected by the administration of ethanol (Fig. 3). Rats given 0.1 ml/100 g ethanol drank 11.4±2 ml water over 6 hr, while rats given glucose solution drank 11.6±2.6 ml water.

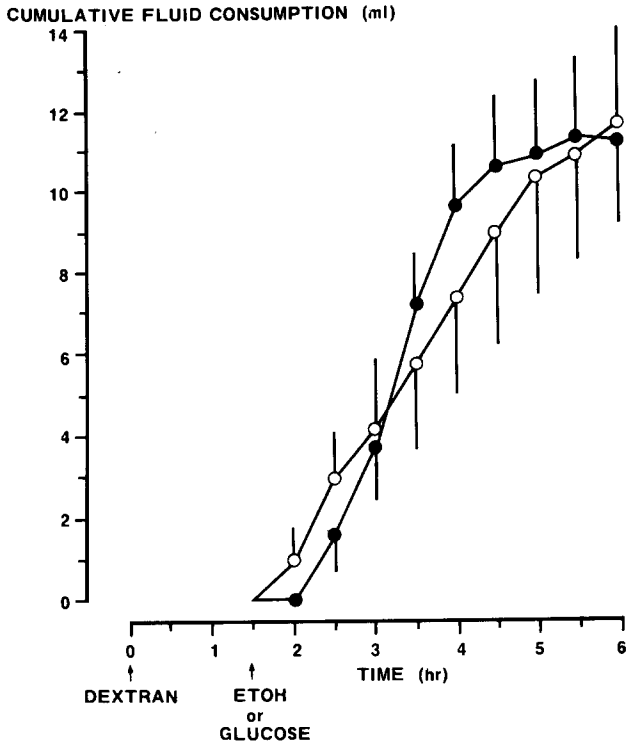

FIG. 3. Cumulative fluid consumption (mean±S.E.) for 6 hr after intraperitoneal injection of dextran in rats administered 0.1 ml/100 g ethanol (●, n=6) and isocalorific glucose (○, n=6). Ethanol had no effect on the drinking response to intraperitoneally administered dextran.

DISCUSSION

These results demonstrate that ethanol inhibits the drinking response to subcutaneous injection of hypertonic saline in the rat, and that this effect is dose-dependent with higher doses having a greater inhibitory effect. This action is independent of the calorific content of ethanol as indicated by the presence of a drinking response to hypertonic saline that was not modified by isocalorific administration of glucose. Further, this action is independent of nonspecific depressant effects of ethanol on the nervous system as indicated by the lack of effect of ethanol on drinking induced by intraperitoneal injection of dextran.

Systemic administration of hypertonic saline elicits drinking by depletion of the intracellular fluid compartment [6,16] whereas dextran acts as a hyperoncotic colloid and induces drinking by reduction of the extracellular fluid compartment [8]. Dextran also causes release of histamine in the rat [18], although evidence indicates that this is not involved in the drinking response to dextran [8]. Unimpaired drinking in response to dextran in rats given ethanol suggests that the effect of ethanol may be confined to thirst mediated by intracellular rather than extracellular dehydration. However, whether this effect is specific to drinking induced by sodium chloride or general to all osmotic stimuli was not tested in the present study. Also the drinking response to dextran was approximately two fold greater than that after hypertonic saline. Thus it is possible that an inhibitory effect of ethanol on drinking induced by dextran may have been masked by the greater level of stimulation achieved.

Wayner [17] reported that ethanol selectively inhibits the activity of lateral hypothalamic neurones that display a high degree of sensitivity to changes in sodium concentration and proposed that this was because of the inhibitory effects of ethanol on membrane sodium conductance [9]. Although the exact nature of osmoreception remains unresolved [11,15], available evidence indicates that membrane active transport of sodium and Na,K-ATPase activity may be essential to the receptor mechanism [1]. A number of agents that inhibit this enzyme system, such as glycerol, ethacrynic acid, ouabain and deuterium, also inhibit thirst and vasopressin release [12]. Ethanol has been shown to have inhibitory effects on Na,K-ATPase-mediated ion transport [9] and this may be the basis for its inhibitory effects on vasopressin release [3] and sodium chloride induced drinking as demonstrated in the study reported here.

In summary, the present results demonstrate that ethanol inhibits salt-load-elicited drinking in the rat. Further, this effect was shown to be dose-dependent and confined to drinking induced by subcutaneous injection of hypertonic saline as compared to intraperitoneal injection of dextran.

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