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Is Alcohol Induced Poikilothermia Mediated by 5-HT and Catecholamine Receptors or by Ionic Set-Point Mechanism in the Brain?

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MYERS, R. D. AND W. D. RUWE. *Is alcohol induced poikilothermia mediated by 5-HT and catecholamine receptors or by ionic set-point mechanism in the brain?* PHARMAC. BIOCHEM. BEHAV. **16**(2) 321–327, 1982.—In adult male Sprague-Dawley rats, stainless steel guide cannulae were implanted stereotaxically in either the lateral or third cerebral ventricle. Postoperatively, each animal was maintained at an ambient temperature of 22°C. Just prior to the intragastric gavage of 4.0 g/kg ethyl alcohol (20% solution) individual animals were fitted with a colonic thermistor probe. Then, control CSF, a monoaminergic receptor antagonist, or a Ca^{++} ion chelating agent, EGTA, was infused into either the lateral or third ventricle (ICV) in a volume of 10.0 μl. Phentolamine (20.0 μg), butaclamol (10.0 μg), or methysergide (20.0 μg) injected ICV all failed to prevent the thermolytic action of alcohol. The fall of 1.5 to 2.0°C in the rat's colonic temperature, ordinarily caused by alcohol, was the same as that without the antagonists and lasted 3.0 to 4.0 hrs. EGTA infused into the rat's lateral cerebral ventricle also did not interfere with alcohol's poikilothermic action. However, EGTA infused into the third cerebral ventricle completely blocked alcohol's effect in lowering the body temperature of the rat. These results suggest that: (1) alcohol's profound effects on body temperature are not mediated by 5-HT, norepinephrine or dopamine pathways which are thought to underlie the mechanisms in the hypothalamus for thermoregulation; and (2) the temperature set-point mechanism, controlled by the ratio of diencephalic Na^+ to Ca^{++} ions, is incapacitated by alcohol. Restoration of the ratio in the diencephalon by the direct, local chelation of Ca^{++} ions thus eliminates alcohol's deleterious effects on body temperature.

5-HT Calcium-sodium ratio Norepinephrine Temperature set-point Dopamine
Alcohol poikilothermia Thermoregulation Ethanol hypothermia

ALCOHOL given systemically in a sufficiently high dose produces a poikilothermia and cannot be considered either as a hypothermic or hyperthermic drug [26]. That is, the ambient temperature at which the animal is maintained determines whether its body temperature rises or falls following a thermolytic dose of alcohol, i.e., 4.0 g/kg. In fact, the body temperature of the alcohol-treated rat can be precisely controlled simply by a systematic variation in air temperature. In addition, the poikilothermia produced by alcohol is identical functionally to that induced by a barbiturate anesthetic in terms of the response to a fluctuation in air temperature [26].

Certain drugs are thought to exert their central effect on temperature by way of an action on a monoaminergic or ionic mechanism [25]. For example, serotonergic pathways in the CNS have been implicated in two of the central actions of ethyl alcohol: (1) the fall in temperature produced by alcohol at room temperature [35,36]; and (2) the tolerance to alcohol as measured by the change in magnitude of a temperature decline [18]. In relation to this, considerable evidence has accrued that the control of body temperature is brought about neurochemically by a balance in the synaptic activity of endogenous monoamines present in the anterior hypothalamus [9,25]. Serotonin (5-HT) is envisaged as the putative neurotransmitter which activates the efferent pathway for heat production [31], whereas the catecholamines, norepinephrine (NE) and dopamine (DA), apparently mediate the neuronal pathways for heat loss [39].

A functional mechanism has been proposed to account for the temperature set-point which is anatomically independent of the monoaminergic thermoregulatory system [1,25]. Based on a large number of pharmacological, anatomical and physiological experiments carried out in the 1970's [25], the theory has evolved that an established reference or set-point temperature of 37°C in the mammal is maintained by an inborn, steady-state ratio between Na^+ and Ca^{++} ions in the posterior hypothalamus [27]. Essentially, if Na^+ ions predominate in the caudal hypothalamic region, the set-point

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shifts upward and body temperature rises. Alternatively, if Ca^{++} ions predominate extracellularly, the reference temperature is re-set downward and the animal's temperature falls.

Within the framework of the monoamine regulatory and ionic set-point theories of temperature control, two sets of experiments were designed. In the first, we determined whether the poikilothermic action of alcohol on the body temperature of the rat is mediated by a monoaminergic receptor system in the brain. Central serotonergic or catecholaminergic receptors were blocked by respective antagonists given into the cerebral ventricle (ICV) just prior to the administration of a poikilothermic dose of alcohol. In the second set, the possibility was examined that essential cations in the brain-stem are involved in alcohol's poikilothermic effect. By an ICV infusion of a Ca^{++} ion chelating agent, it was determined whether the change in the body temperature of the rat would be either enhanced or prevented by an induced imbalance in the brain's Na^{+}-Ca^{++} ratio [28].

METHOD

Following surgical procedures described elsewhere [33], 20 gauge thin-walled stainless steel guide tubes were stereotaxically positioned bilaterally just above the lateral cerebral ventricle in eight rats given 35 mg/kg sodium pentobarbital intraperitoneally. In four other rats, a single cannula was implanted just dorsal to the third ventricle using the same surgical procedures. Each cannula was cut to a length of 15 mm, beveled at a 45° angle at the tip and fitted with a 23 gauge stylet of a corresponding length and bevel. The stereotaxic coordinates for the lateral ventricle guides were AP 6.2, lateral 1.5, and horizontal −2.6 below dura mater, and for the third ventricle guide tube, AP 6.4, lateral 0.0, and horizontal −6.0 below the dura.

Intraventricular Infusions

To inject the receptor antagonist or control vehicle into the ventricular lumen, a 23 gauge injector needle was attached to one end of a length of PE 50 intramedic tubing. After the other end was affixed to a syringe filled with a receptor antagonist, chelator or control vehicle, the tubing was then filled with the respective solution. Inflow into the ventricle by gravity was, in each case, established by adjusting the length of each injector needle to 1.0 mm or more below the tip of the guide tude according to patency procedures described previously [33]. Following the intraventricular infusion of a volume of 10.0 μl over approximately 10 to 30 sec, the injector needle was kept in place for an additional 30 sec, removed, then replaced by the indwelling stylet.

Receptor Anatagonists and Chelating Agent

The following compounds were used: phentolamine hydrochloride (Ciba) in a dose of 20 μg/10 μl; D-butaclamol HCl (Ayerst) in a dose of 10 μg/10 μl; methysergide maleate (Sandoz) given in a dose of 20 μg/10 μl; and the Ca^{++} ion chelating agent, ethyleneglycolbis-(β-amino ethyl ether) N,N′-tetra-acetic acid (EGTA) [40] given in a dose of either 4.0 or 8.0 μg/10 μl. The doses of the first three compounds are expressed in terms of the base and were selected on the basis of optimal doses which block either a serotonergic or catecholaminergic fall in temperature or other physiological responses when the receptor agonist and antagonist are given by the intracerebroventricular route [2, 5, 6, 13].

EGTA (Sigma) was infused into the third ventricle of four animals in both the 4.0 μg and 8.0 μg doses but was given in the lateral ventricle in the 4.0 μg dose to four rats and in the 8.0 μg dose to the remaining four. The microgram concentrations selected are effective intraventricularly in inducing a rise in body temperature as well as in counteracting the effects of a general anesthetic [28].

The Ca^{++} ion chelating agent and the receptor antagonists were dissolved in an artificial CSF containing five ions [22] in the following concentrations: Na^{+}, 127.6 mM; K^{+}, 2.5 mM; Ca^{++}, 1.3 mM; Mg^{++}, 1.0 mM; and Cl^{-}, 134.5 mM. Each solution was prepared pyrogen-free with glass-distilled water and passed through a 0.22 μm Swinnex millipore filter. The tubing, injector needles, and other materials were always stored in 70% alcohol and flushed repeatedly prior to use.

Test Procedures

After each animal was fitted with a YSI 401 Thermistor probe inserted into the colon to a depth of 4.0–6.0 cm, the body temperature of the rat was monitored for a baseline period of at least 1.5 hrs [33]. The stylet was removed and the solution of methysergide, phentolamine, butaclamol or EGTA was administered either in the lateral or third cerebral ventricle. If there was evidence of occlusion as reflected by sluggish inflow or a reflux of the solution, the experiment for that animal was terminated. After an interval of 20–30 min had elapsed following the ventricular injection, a constant dose of 4.0 g/kg alcohol was given in a 20% concentration by intragastric gavage to all of the rats according to the procedures described by Myers [26]. With the ambient temperature of the experimental room maintained at 22°C±1.0°C, this assured a decline in the rat's body temperature of 1.5°C to 2.0°C.

After the experiments had been completed, patency of the cerebral ventricle of each rat was verified in one of two ways [24]. Either angiotensin was infused into the ventricle in a dose of 100 ng/10 μl to induce spontaneous drinking in the water-replete rat, or each brain was fixed in Formalin, sectioned on a cryostat, mounted and examined for the position of the respective ventricular cannulae [21]. Figure 1 presents a representative section that portrays the penetration of ventricles by the lateral ventricular cannulae.

RESULTS

When the rat is kept at an ambient temperature of 22°C, an intragastric gavage of 8.0–12.0 ml of 0.9% saline exerts virtually no effect on its body temperature. However, as shown in Fig. 2, an intragastric dose of 4.0 g/kg of alcohol (ETOH) given in a corresponding volume induced a fall in temperature of 1.5°C to 2.0°C of 3 to 4 hrs duration.

Monoamine Antagonist's Effect on Alcohol Poikilothermia

No significant differences were found between the results obtained when the receptor antagonists were injected into the third ventricle as compared with the lateral cerebral ventricle, $t(17)=0.56$. Therefore, the data were pooled for both groups. Figure 3 (top) shows that following the intraventricular injection of 20 μg of the α-adrenergic antagonist, phentolamine (PHT), little or no change in body temperature occurred. When alcohol (ETOH) was given intragastrically

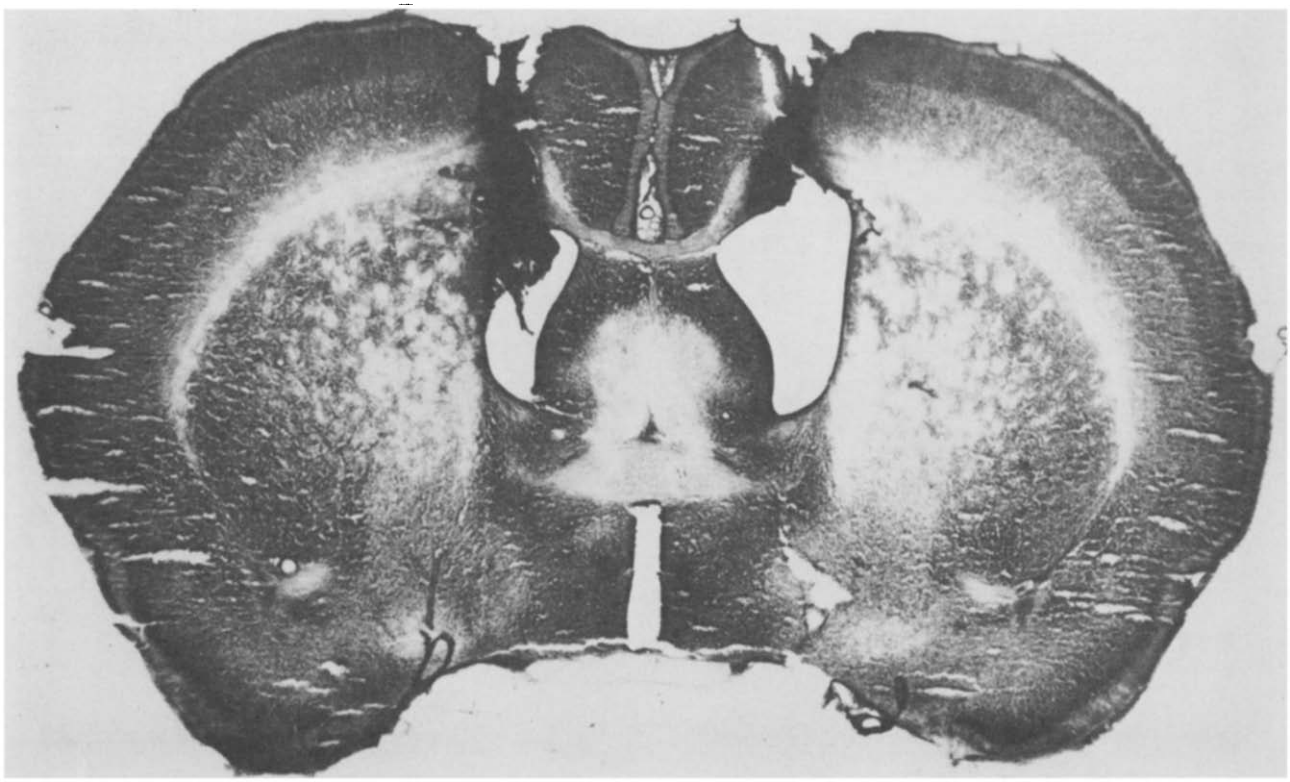

FIG. 1. Representative histological section of rat's brain cut in coronal plane at 75 microns and stained with cresyl violet. The lesion tracks produced by the two cannulae, positioned bilaterally, are depicted by the black columns above the ventricles.

to the rats in a dose of 4.0 g/kg at an ambient temperature of 22°C, following the phentolamine injection, the characteristic long-lasting and intense temperature decline was produced. The response was virtually identical to that produced by alcohol given alone (Fig. 2) at a room temperature of 22°C. The nadir of the fall in body temperature reached nearly 2.0°C during the course of the 3.0 hr period following the alcohol gavage.

When 10 μg of the dopaminergic receptor antagonist, butaclamol, was infused into the ventricle, there was virtually no effect on the rat's body temperature. As presented in Fig. 4, when the same dose was given just prior to the intragastric administration of 4.0 g/kg alcohol, again the fall in temperature was just as marked as that produced by alcohol alone (Fig. 2).

Figure 5 shows that after the intraventricular injection of 20 μg of the serotonergic receptor antagonist, methysergide, a small but gradual decline in the rat's temperature ensued over the next 2.0 hr which exceeded 0.5°C. Such a response could be expected if the 5-HT neurons underlying heat production [25] were partially blocked. The 4.0 g/kg dose of alcohol given by gavage following methysergide pretreatment nevertheless induced a profound fall in the rats' body temperature, protrayed in Fig. 5, the nadir of which approximated 1.5°C. In comparison to the control results, this represents a slight attenuation of the alcohol hypothermia which was not statistically significant ($p>0.10$).

As shown in Table 1, EGTA injected in a dose of either 4.0 or 8.0 μg into the lateral cerebral ventricle of the rat failed to

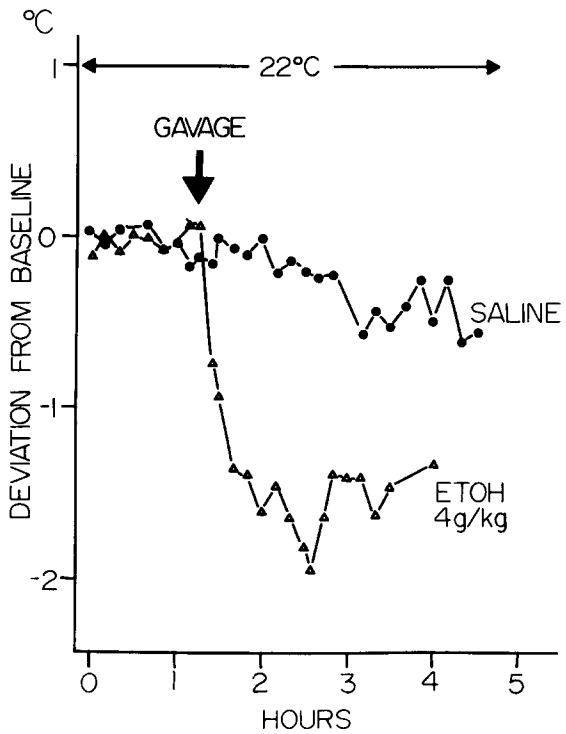

FIG. 2. Deviation from baseline in °C in colonic temperature of the rat kept at an ambient temperature of 22°C. At the arrow, either 0.9% saline or 4.0 g/kg alcohol (ETOH) was infused (isovolumetrically) by gavage into the rat's stomach (n=6). Time in hrs on abscissa (modified after Myers [26]).

TABLE 1

CHANGE IN BODY TEMPERATURE, EXPRESSED IN °C DEVIATION FROM BASELINE, RECORDED AT 0 TIME OF ALCOHOL (ETOH) GAVAGE AND AT 30 MIN INTERVALS THEREAFTER

	Time in Minutes						
	30	60	90	120	150	180	Mean
EGTA (4.0 μg) plus 4.0 g/kg ETOH (n=8)	−0.8	−1.4	−1.9	−2.4	−2.5	−2.4	−1.6 ± 0.38
EGTA (8.0 μg) plus 4.0 g/kg ETOH (n=4)	−1.4	−1.6	−1.8	−1.5	−1.6	−1.5	−1.4 ± 0.19
ETOH (4.0 g/kg) alone (n=4)	−0.8	−1.6	−2.0	−1.5	−1.7	−1.4	−1.3 ± 0.24
Saline Control (n=6)	−0.1	−0.1	−0.1	−0.2	−0.4	−0.3	−0.2 ± 0.05

Mean change ± S.E. is presented in the far right column for each of the three treatment groups and for the control rats in which saline gavage was administered. EGTA injected into lateral ventricle.

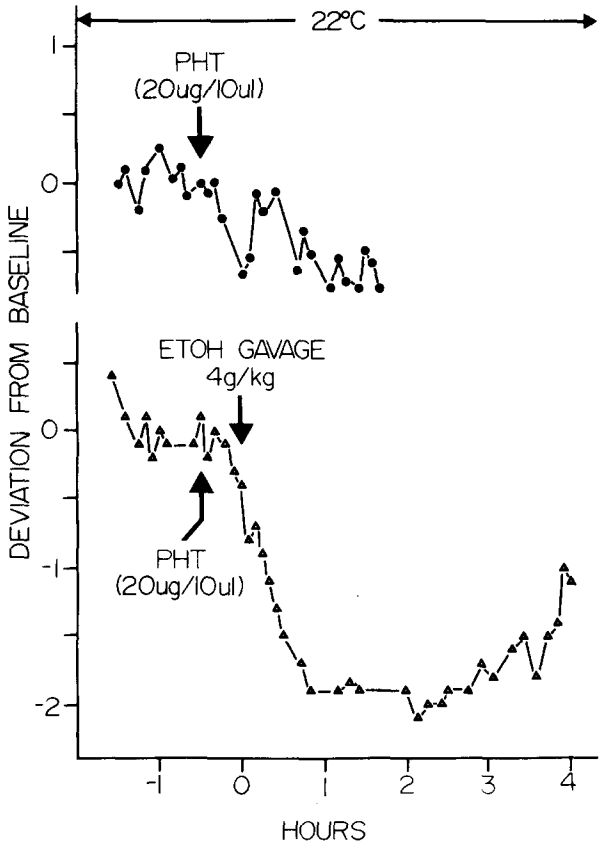

FIG. 3. Deviation from baseline in °C in colonic temperature of the rat kept at an ambient temperature of 22°C. Top: at arrow, phentolamine (PHT) injected ICV in a dose of 20.0 μg in a volume of 10 μl (n=4). Bottom: at first arrow, PHT injected ICV, followed 30 min later (second arrow) by intragastric gavage of 4.0 g/kg alcohol (ETOH) (n=4).

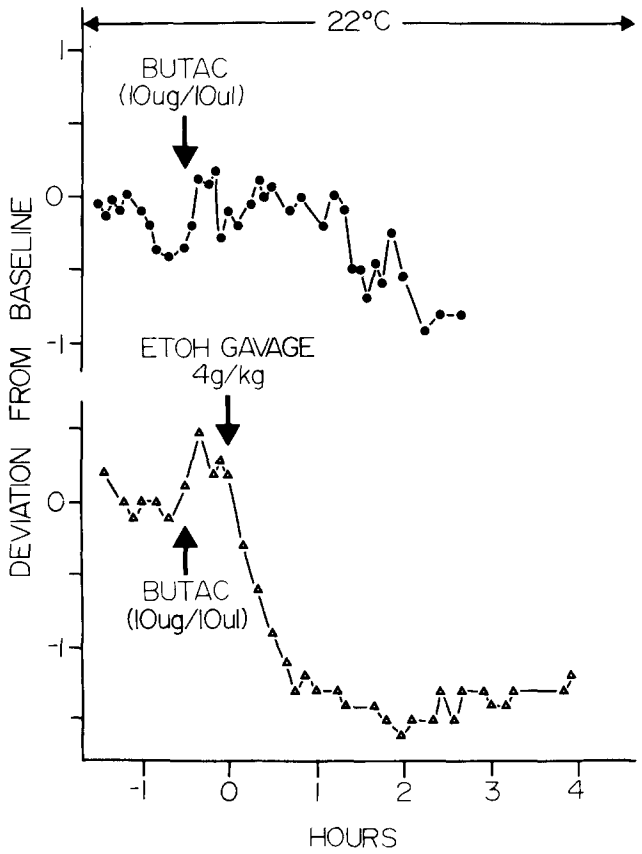

FIG. 4. Deviation from baseline in °C in colonic temperature of the rat kept at an ambient temperature of 22°C. Top: at arrow, butaclamol (BUTAC) injected ICV in a dose of 10.0 μg in a volume of 10 μl (n=4). Bottom: at first arrow, BUTAC injected ICV, followed 30 min later (second arrow) by intragastric gavage of 4.0 g/kg alcohol (ETOH) (n=4).

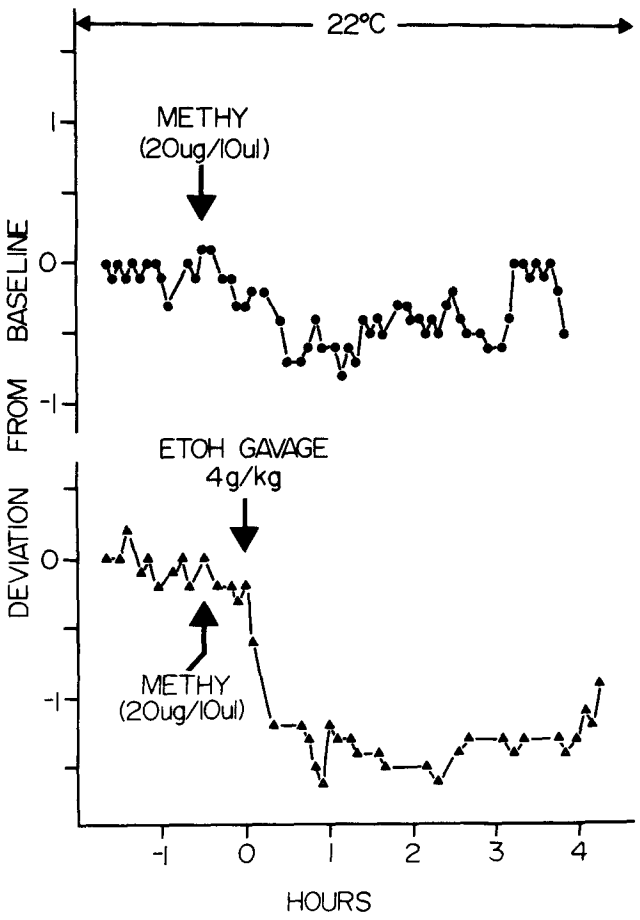

FIG. 5. Deviation from baseline in °C in colonic temperature of the rat kept at an ambient temperature of 22°C. Top: at arrow, methysergide (METHY) injected ICV in a dose of 20.0 μg in a volume of 10 μl (n=4). Bottom: at first arrow, METHY injected ICV, followed 30 min later (second arrow) by intragastric gavage of 4.0 g/kg alcohol (ETOH) (n=4).

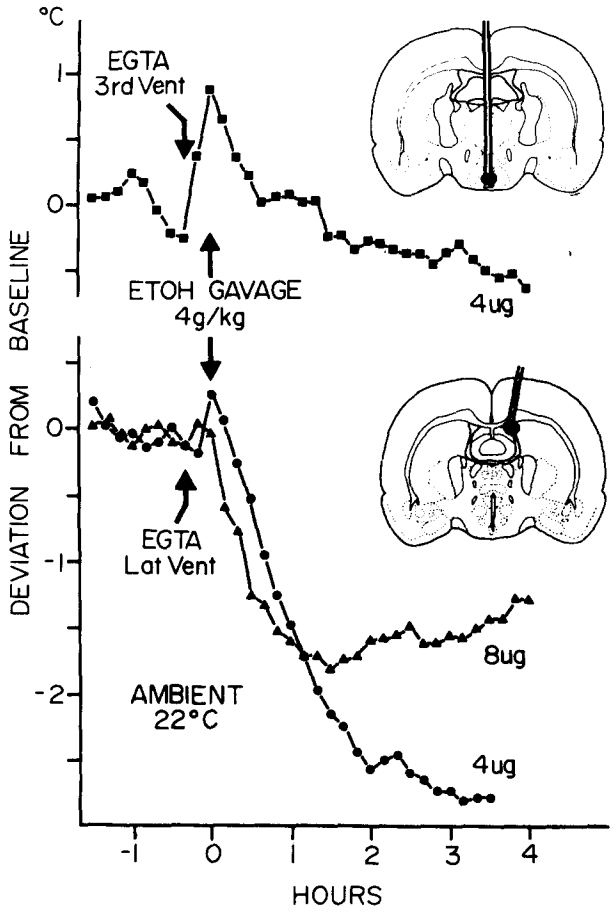

FIG. 6. Deviation from baseline in °C in colonic temperature of the rat kept at an ambient temperature of 22°C. Top: at first arrow, 4.0 μg EGTA infused into the third ventricle (histological inset) followed, at second arrow, by intragastric gavage of 4.0 g/kg alcohol (ETOH) (n=4). Bottom: at first arrow, 4.0 μg (n=4) or 8.0 μg (n=4) EGTA infused into lateral ventricle (histological inset) followed, at second arrow, by intragastric gavage of 4.0 g/kg alcohol (ETOH) (n=4). Time in hrs on abscissa.

prevent the intense decline in temperature produced by the 4.0 g/kg alcohol gavage. However, following the infusion of EGTA into the third cerebral ventricle, the temperature of the rats began to rise immediately as expected [28]. The intragastric administration of 4.0 g/kg alcohol 30 min after EGTA in the third ventricle reversed the rise in temperature within 5–10 min, as shown in Fig. 6 (top). There was no evidence whatsoever of the typical poikilothermia arising subsequent to the alcohol treatment. In fact, the temperature of the animals simply returned to the previous baseline level. Conversely, EGTA infused into the lateral cerebral ventricle, even in the higher 8.0 μg dose, did not prevent the sharp alcohol-induced fall in the animals's body temperature. As depicted in Fig. 6 (bottom), the higher dose of EGTA did, however, tend to retard the depth of the hypothermia, in comparison to the 4.0 μg dose, but the difference was not significant.

DISCUSSION

It has been proposed that the regulating system for body temperature is subserved by monoaminergic neurons located in the rostral hypothalamus [9]. This mechanism, the balance in synaptic release of the monoamines, is hypothesized to be responsible for the physiological defense and consequent survival of the animal against the heat or cold [25]. The amine pathways which they form are thought to be functionally and anatomically independent of the ionic mechanism which controls the set-point for body temperature [25]. The present results suggest that indole- and catecholamine containing neurons in the brain-stem, which are affected by pharmacological receptor antagonists injected into the ventricular spaces [8,32], are not involved in the poikilothermic action of alcohol. In fact, when alcohol is given to a rat kept at an ambient temperature of 22°C, the intensity of the rat's decline in body temperature is essen-

tially the same as that seen in the absence of a blocking agent. It is important to note that 22°C ambient level constitutes a cold challenge because it is below the animal's euthermic temperature.

Thus, alcohol's poikilothermic action apparently is not mediated by a neurotransmitter mechanism in the diencephalon postulated for heat production and heat dissipation. Corresponding to the passive, thermolabile reaction of the rat to external heating or cooling following alcohol, it thus seems likely that alcohol alters body temperature independently of the central thermoregulatory process.

An alternative explanation is that the set-point for the animal's temperature is incapacitated or re-set by alcohol [19]. This would seem plausible in light of the fact that a high dose of alcohol can cause death in the subject exposed to either extreme of hot or cold temperature [11,12]. Further, a drug which affects a monoamine in the CNS has little or no effect on the preference for a warm ambient temperature seen during withdrawal from alcohol [3] and its associated hypothermia.

Ten years ago it was demonstrated that an alteration in the ratio of Na^+ to Ca^{++} ions in the animal's cerebral ventricle, in favor of Na^+, decreases the depth of anesthesia markedly [28]. EGTA infused ICV in the rat anesthetized with either urethane or a barbiturate not only reverses the effect of the anesthetic, but augments the animal's respiratory rate and motor activity subsequently [28]. In this case, the chelation of Ca^{++} ions artifically restores the drug-impaired set-point temperature by functionally re-establishing the normal, steady state ratio of Na^+ to Ca^{++} ions. The extent of the effect on the set-point depends on the dose of EGTA as well as on the anatomical locus of its application. For example, an excess in cation concentration shifts an animal's temperature set-point by a specific action at circumscribed sites in the diencephalon within the posterior hypothalamus [29, 30, 34]. Because the present results with EGTA are consistent with the findings obtained previously, the anatomical region where the chelation of Ca^{++} ions prevents poikilothermia and set-point impairment produced by alcohol is most likely the hypothalamus.

Although the inhibition by systemic pCPA of 5-HT synthesis can enhance alcohol hypothermia, the peripheral effect of the drug on vasomotor tone and other autonomic function probably is responsible [35]. The β-adrenoreceptor blocker, propranolol, given systemically reduces sleep-time after alcohol treatment [20]. However, other systemically given compounds, including amphetamine and bemegride, which also alter the level of monoamines in the CNS fail to alter the immediate thermolytic effect of alcohol [17]. The effects of alcohol over a longer term in regard to the development of tolerance, however, may be mediated by serotonergic neurons [18].

The involvement of calcium in the local action of alcohol on the brain has long been recognized [37]. The fall in temperature caused by alcohol given to a rat at room temperature is enhanced by excess Ca^{++} ions given ICV [7,14] presumably because of calcium's well-known hypothermic effect [23]. In an *in vitro* preparation of the rat's brain, alcohol in a pharmacological concentration inhibits the uptake of Ca^{++} ions by synaptosomes [15]. Further, the same 4.0 g/kg dose used in the present experiments reduces the concentration of Ca^{++} ions in the hypothalamus and other areas of the brain-stem [10]. Since alcohol or other anesthetic may inhibit the release of neurotransmitters *in vitro* [4,16], this effect may be due to the suppression of Ca^{++} influx into the nerve cell or other vital process in which the cation is involved [38].

Taken together, these observations suggest that the chelation of Ca^{++} ions by EGTA prevents alcohol-induced poikilothermia by one or more mechanisms which include: (a) the de-stabilization of the nerve cell membrane [38]; (b) an alteration in the ratio of Na^+ to Ca^{++} ions in favor of Na^+ [28]; or (c) an enhanced availability of Ca^{++} ions to cells in the brain-stem. Thus, it is possible that the acitivty of a neurotransmitter in the heat production pathway, which is suppressed by alcohol, would be reinstated by the available Ca^{++} ions. Consequently, the animal would retain its heat producing capacity after alcohol loading and hence maintain its normothermic balance [34].

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