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Effect of Gaba-Transminase Inhibition on Rectal Temperature in Mice

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SCHECHTER, P. J. AND Y. TRANIER. *Effect of GABA-transaminase inhibition on rectal temperature in mice.* PHARMAC. BIOCHEM. BEHAV. 6(6) 667–669, 1977. – γ-Acetylenic GABA (amino-4-hex-5-ynoic acid) and γ-vinyl GABA (amino-4-hex-5-enoic acid), two catalytic irreversible inhibitors of GABA-transaminase, produce marked sustained elevations in brain GABA concentrations. Associated with these biochemical changes is a decrease in the rectal temperature of mice. This hypothermia can be reversed by increasing ambient temperature. The results suggest GABA plays an important role in mammalian central thermoregulation.

γ-Acetylenic GABA γ-Vinyl GABA GABA-transaminase inhibition
Thermoregulation

IT IS WELL established that monoamine neurotransmitters influence central thermoregulation, (recently reviewed by Hellon [8]). The role of γ-aminobutyric acid (GABA), probably the principal inhibitory substance in mammalian CNS, on central control of body temperature is less clear. Administration of GABA directly into the brain or into the ventricular system has been reported to both decrease [18] or to increase body temperature in rats [3], or to do either depending on experimental design [6]; to produce hyperthermia at low doses and variable results on body temperature at high doses in the dog [5]; and to produce hyperthermia in neonatal rabbits [11]. Allyglycine, which is an inhibitor of glutamic acid decarboxylase and thus decreases brain GABA [1], produces a hypothermic response in rats [14]. The hypothermic effects of desipramine have been attributed to its ability to increase brain GABA levels in mice [16]. Furthermore, imidazoleacetic acid, a potent activator of postsynaptic GABA receptors [13], decreases body temperature of mice following intraperitoneal administration [19].

Two catalytic irreversible inhibitors of GABA-transaminase (GABA–T; γ-aminobutyric acid-α-ketoglutarate transaminase), the enzyme responsible for GABA catabolism, have been synthesized in our research center [9]. Since catalytic inhibitors use the enzyme's own mechanism to precipitate its irreversible inhibition [17] they are quite specific. These compounds, γ-acetylenic GABA and γ-vinyl GABA, represent the first known catalytic GABA–T inhibitors which effectively increase brain GABA concentrations when administered by a peripheral route [9]. They enabled us to investigate the effect of increased brain GABA levels on body temperature in mice, without the necessity of intracranial manipulations.

METHOD

Rectal temperatures of 48 CD_1 mice (Charles River, France), body weight 25–31 g, were determined using an Ellab Thermomètre Universal fitted with a rectal probe. Mice were then randomly distributed into six equal groups. Two groups were injected IP with γ-acetylenic GABA (amino-4-hex-5-ynoic acid, RMI 71645) 100 mg/kg, two groups with γ-vinyl GABA (amino-4-hex-5-enoic acid, RMI 71754) 1500 mg/kg (both products administered as aqueous solutions, 1 ml/100 g body weight), and two groups with an equal volume of saline. These doses of the GABA analogs are approximately equipotent in protecting against audiogenic seizures and in depressing spontaneous motor activity (unpublished observations). All injections were done between 9:00 a.m. and 10:00 a.m. One group from each of the treatments was placed into a compartmentalized heated box maintained at a constant temperature of 34 ± 1°C. The remaining three groups were placed in an identical box with the heating apparatus turned off (22 ± 1°C). Rectal temperatures were measured at various times postinjection.

To insure that an alteration in ambient temperature did not itself influence the brain levels of GABA or the effects of the two enzyme inhibitors on GABA metabolism, a parallel experiment was done. Brain GABA concentrations were determined in six additional groups of 6 mice with treatments and temperature conditions identical to the above experiment. At 4 hr after injection the mice were sacrificed by decapitation and the brains rapidly removed and frozen in liquid nitrogen within 30 sec after sacrifice. To determine whole brain GABA concentrations the frozen brains were weighed and homogenized in 4 volumes of chilled 15% trichloroacetic acid. The homogenate was centrifuged for 20 min at 2000 g at 4°C and the precipitate was extracted three times with 1 ml of water. The supernatant and washings were pooled and again centrifuged, and the GABA content of the final supernatant was determined using a Beckman Amino Acid Analyzer [10,15].

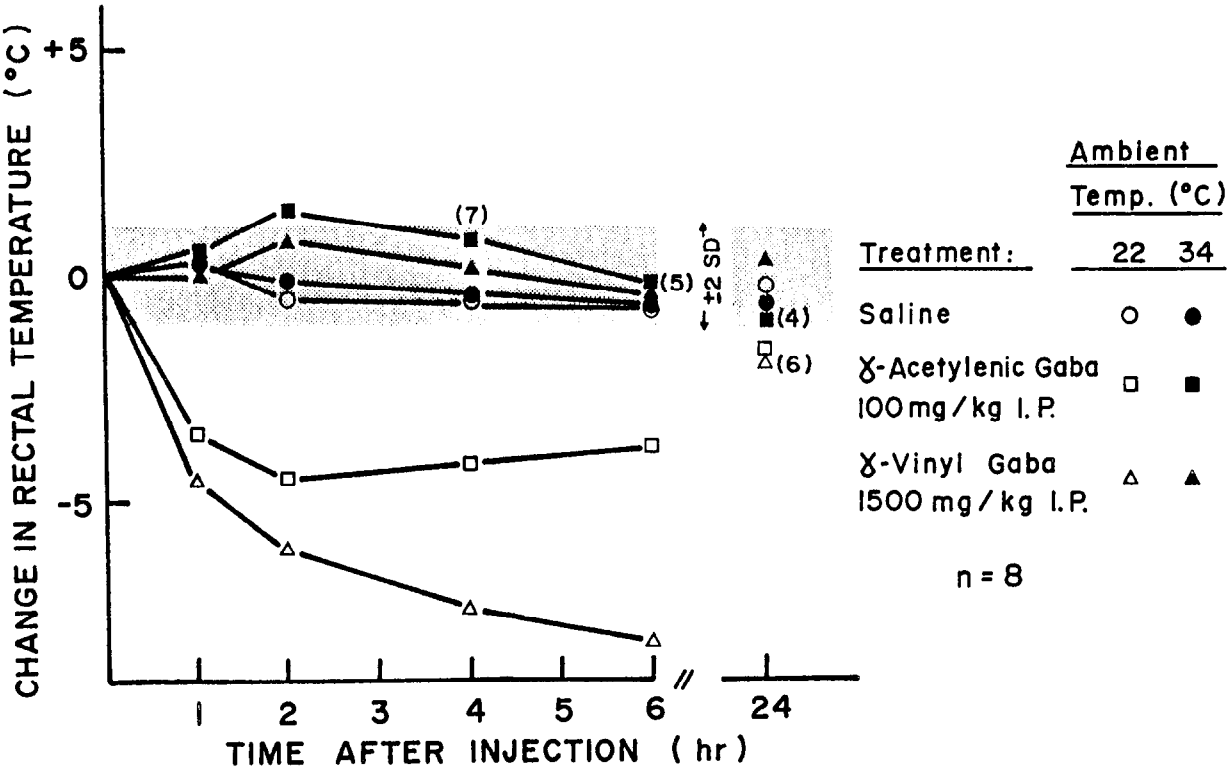

FIG. 1. Effect of γ-acetylenic GABA, 100 mg/kg IP, and γ-vinyl GABA, 1500 mg/kg IP, on rectal temperature in mice maintained at ambient temperatures of 22° and 34° as a function of time after injection. Eight animals were used per point unless otherwise noted in parentheses. Shaded area represents ± 2 SD of pretreatment values.

RESULTS

Initial mean rectal temperature of 48 mice was 38.0°C (SD = 0.57°C) (Fig. 1). The mean temperature of saline-injected controls at 22°C and at 34°C remained within two standard deviations of the initial temperatures throughout the course of the experiment. On the other hand, mice injected with γ-acetylenic GABA, 100 mg/kg IP, and maintained at 22° showed a marked decrease in body temperature within 1 hr after treatment with a depression of about 4° lasting at least 6 hr. A more profound drop in body temperature was seen in mice at 22° following treatment with γ-vinyl GABA, 1500 mg/kg; by 6 hr body temperature had been decreased to about 30°. In both cases body temperature approached control values 24 hr post-treatment. When ambient temperature was maintained at 34°, the hypothermia induced by γ-acetylenic and γ-vinyl GABA was prevented. Mice treated with γ-acetylenic GABA and maintained at 34° exhibited a higher rate of mortality (50% by 24 hr) than those maintained at 22° (0% by 6 hr; 25% by 24 hr).

Both γ-acetylenic GABA and γ-vinyl GABA increased whole brain GABA concentrations (Table 1). γ-Acetylenic GABA, 100 mg/kg IP, produced approximately a 400% increase over control at 4 hr after injection and γ-vinyl GABA, 1500 mg/kg IP, a 500% increase. No significant differences were found in control brain GABA concentrations or in the increase in GABA produced by either compound between ambient temperatures of 22° and 34°.

TABLE 1

INFLUENCE OF DIFFERENT AMBIENT TEMPERATURES ON WHOLE BRAIN GABA CONCENTRATIONS AND RESPONSE TO GABA-TRANSAMINASE INHIBITORS IN MICE (4 HR POST-TREATMENT)

TREATMENT	WHOLE BRAIN GABA CONCENTRATION (μG/G) (MEAN ± SEM ; N = 6)	
	22°C	34°C
SALINE	220.3 ± 18.4	223.2 ± 7.9
γ-ACETYLENIC GABA 100 MG/KG	1136.8 ± 38.0	1282.3 ± 66.8
γ-VINYL GABA 1500 MG/KG	1373.4 ± 55.7	1506.3 ± 35.7

DISCUSSION

γ-Acetylenic and γ-vinyl GABA, two irreversible GABA–T inhibitors which cause elevations in brain GABA concentrations, also decrease body temperature in mice when given by a peripheral route. This hypothermic effect can be eliminated by raising the ambient temperature to 34°C.

It can be assumed that these GABA analogs exert their hypothermic effect via a central mechanism, and specifically via increases in brain GABA produced by GABA–T inhibition, for the following reasons: γ-acetylenic and γ-vinyl GABA have been shown to be relatively specific for GABA–T in vivo. They show little or no activity against alanine or aspartate transaminase [9,10]. Neither compound effects vasomotor tone at the doses used

(unpublished observations). In mammals GABA, as well as the enzymes which synthesize and catabolize it, exist almost exclusively in the CNS and retina with only traces found in peripheral nerve and other tissues [4]. Since GABA does not easily transverse the blood-brain barrier [20], inhibition of its catabolism should increase its concentration almost exclusively in the CNS. Furthermore, we found that the intraperitoneal administration of GABA in doses up to 1 g/kg failed to influence body temperature in mice (unpublished observations).

Modification of the hypothermic action of a neurotransmitter by an increase in the ambient temperature has been thought to indicate that the neurotransmitter in question acts as an inhibitor of temperature regulation pathways, i.e., the neurotransmitter can only inhibit a pathway that normally functions to maintain or increase heat production [2]. Using this concept, elevated brain GABA concentrations would inhibit neural pathways driving heat production at the lower temperature but would have no effect when environmental temperature was raised. This is consistent with the inhibitor roles attributed to GABA in other CNS functions [12].

The regional or subcellular distribution within the CNS of the increased GABA achieved after GABA–T inhibition is not yet known. GABA–T and GABA are found in essentially all areas of the brain and spinal cord with relatively high values reported in the hypothalamus [7], the brain region considered to play the major role in thermoregulation. Hence, it seems safe to assume that GABA–T inhibition augments the GABA concentration in the hypothalamus.

It is clear that interactions exist between GABA and other neurotransmitters in the central nervous system. Alterations in brain GABA have been shown to influence catecholamine and serotonin [21]. Whether the effects of elevated brain GABA on body temperature are direct effects of GABA on temperature control mechanisms or indirect effects via a GABA modulation of neural pathways mediated by other neurotransmitters is not established. However, from previous work and from our results it is likely that GABA does play a role in central thermoregulation.

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