The estradiol released from the implant maintained the weights of the uteri, increased pituitary weights significantly, and had no effect on the adrenal weights (Table V). Five of the eight uteri from the estrogen-treated rats were distended with fluid at autopsy—further evidence of unopposed estrogen action. All of these findings confirm that the implants were able to maintain a physiologically significant level of estradiol in the treated rats.

No inflammations were observed after 8 months of subcutaneous residence.

CONCLUSIONS

The diffusion, flux, and solubility coefficients of estrogen are 100, 1000, and 10 times, respectively, higher through silicone than through polyethylene. The values of diffusion coefficients of estrogen through the membranes derived independently by the time lag method and by sorption kinetics were in good agreement. Coating of silicone on polyethylene had no influence whatever on the flux of estrogen through it.

An interesting phenomenon observed during this study was that permeability coefficients through the membranes were orders of magnitude higher when estrogen was present as a solid rather than in solution, while the diffusion coefficients found by the time lag method were equal in both cases. This finding can be explained by the fact that a larger concentration gradient within the membrane is established in the former case partly because the absolute concentration may be higher in the vapor phase above the solid and partly because of larger distribution coefficient in favor of the membrane. The existence of this phenomenon indicates that control of the partial vapor pressure of the encapsulated drug is a powerful method for controlling its release rate.

An implant of desirable size regulating the flux of estrogen through a polyethylene membrane, coated with silicone, was prepared based on these findings. This implant can be successfully used in rats.

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Hypothermic Response following Administration of 2-Amino-4-pentenoic Acid (Allylglycine)

R. L. LILIEDAHL, C. F. RYAN, and R. W. PIEPHO ˣ

Abstract □ Intraperitoneal administration of allylglycine resulted in a hypothermic response in rats. At a dosage of 90 mg/kg, a significant decrease in temperature was noted at 4 and 8 hr after administration, but no significant difference was noted at 12 hr. The same dose administered intraperitoneally to decapitated rats did not result in a hypothermic response, and intraventricular administration resulted in a rapid onset of hypothermia after 1 hr. These findings are indicative of a central site of action for the allylglycine-induced hypothermia. Quantitative assay of hypothalamic monoamines (norepinephrine, serotonin, and dopamine) did not show any significant changes at 4, 8, and 12 hr postadministration when compared to controls. A significant decrease in hypothalamic γ-aminobutyric acid was noted at each of these time points when compared to controls. These data suggest an important role for γ-aminobutyric acid in mammalian thermoregulatory control.

Keyphrases □ Allylglycine (2-amino-4-pentenoic acid)—effect of intraperitoneal and intraventricular administration on hypothermic response in rats □ Hypothermia—effect of intraperitoneal and intraventricular administration of allylglycine, rats □ Thermoregulation—effect of allylglycine intraperitoneal and intraventricular administration on hypothermic response, rats

Feldberg and Myers (1) first proposed that body temperature was regulated by a fine balance in the release of catecholamines and serotonin in the anterior hypothalamus. The hypothesis was based on the responses to intraventricular injection of these agents in the cat. Subsequent investigations showed that there is a distinct species variation in the temperature response, both in magnitude and direction of change, when these agents are administered by the intraventricular route. Feldberg and Lotti (2) showed that intraventricular administration of small doses of norepinephrine in the rat resulted in an increased body temperature, while larger doses had an opposite action. Intraventricular or intracisternal administra-

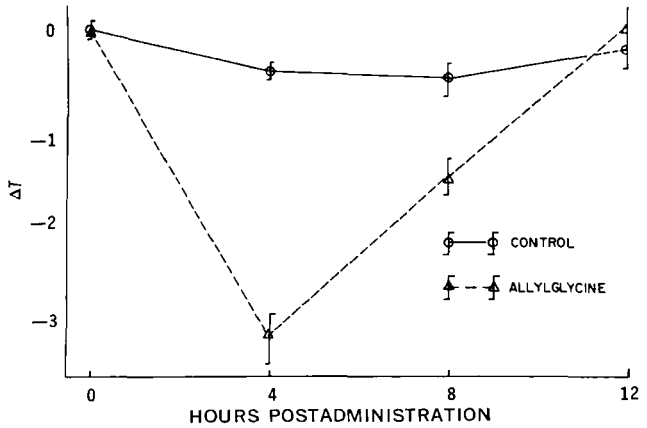

Figure 1—*Rectal temperature changes in unanesthetized rats for control and allylglycine-treated (intraperitoneally) groups. Brackets denote the standard error of the mean for each point* (n = 6).

tion of serotonin results in a decreased body temperature.

In 1971, Sgaragli and Pavan (3) reported that intracisternal administration of γ-aminobutyric acid could also affect thermoregulation by causing a decrease in body temperature. However, a previous study found that γ-aminobutyric acid injected into the hypothalamus in much smaller doses resulted in an increase in body temperature (4). Thus, γ-aminobutyric acid, which is believed to act as an inhibitory neurotransmitter at central synapses (5–7), also appears to play some part in mammalian thermoregulation.

Allylglycine[1] (2-amino-4-pentenoic acid) was shown (8) to possess convulsant activity when administered to rats. Dose–response relationships in mice (9) showed that a dose of 100 mg/kg was fatal to one out of 20 animals tested, while 17 of 20 died at 200 mg/kg. Subsequent studies (10) indicated that allylglycine, administered at a dose of 150 mg/kg to rats, caused an inhibition of glutamic acid decarboxylase and resulted in a 40% decrease in the concentration of γ-aminobutyric acid in the cerebral cortex.

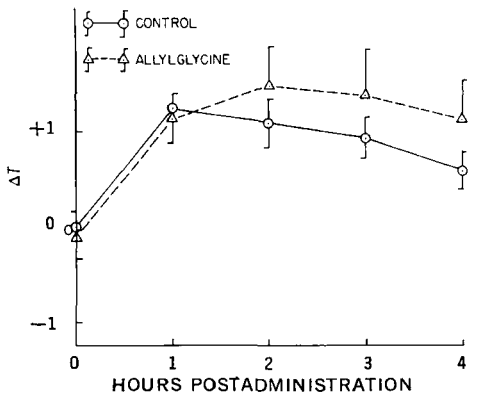

Figure 2—*Rectal temperature changes in decapitated rats for control and allylglycine-treated (intraperitoneally) groups. Brackets denote the standard error of the mean for each point* (n = 4).

[1] Sigma Chemical Co., St. Louis, Mo.

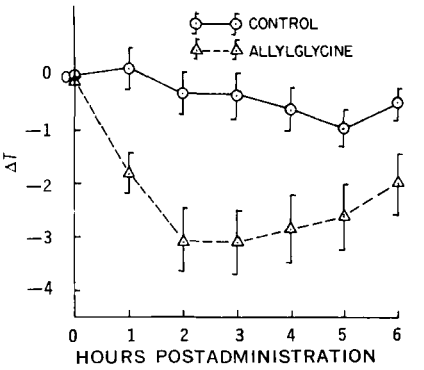

Figure 3—*Rectal temperature changes in unanesthetized rats for control and allylglycine-treated (23 μg intraventricularly) groups. Brackets denote the standard error of the mean for each point* (n = 6).

In light of these known effects of neurotransmitter alteration on thermoregulation, the purposes of this investigation were to: (*a*) administer allylglycine to the rat and study the effects of decreased γ-aminobutyric acid level on thermoregulation, and (*b*) correlate these effects with changes in the central levels of the neurotransmitters believed to be important in thermoregulation—*viz.*, norepinephrine, serotonin, and dopamine.

EXPERIMENTAL

Male albino rats[2], 200–250 g, were housed for 1 week and fasted overnight prior to experimentation. The experiments were conducted over a 12-hr period each day, from 7:30 am to 7:30 pm to avoid problems of circadian fluctuation; such fluctuations in hypothalamic levels of γ-aminobutyric acid are known to occur (11). Rats were housed individually on the day of the experiment to prevent interaction with other rats.

Ambient temperature (23.5 ± 0.5°) was maintained throughout the experiment. The colonic temperature of each rat was recorded every 4 hr over 12 hr *via* a rectal temperature probe[3] inserted 6 cm into the colon, allowing 2 min for adjustment to proper temperature while the animal was restrained in a small plastic cage. All drugs were administered in a volume of 0.1 ml/100 g body weight. Saline solution, 0.9%, served as a control.

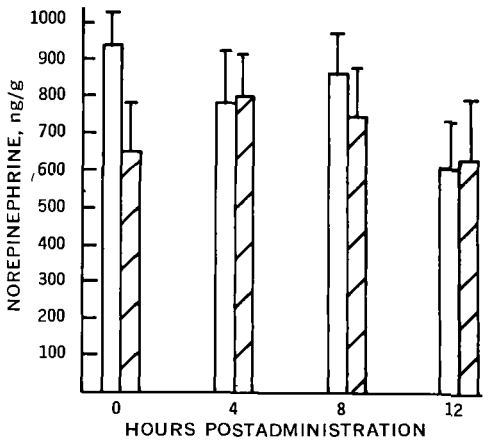

Figure 4—*Level of norepinephrine in the hypothalamus of control (□) and allylglycine-treated (intraperitoneally) (▨) groups. Brackets denote the standard error of the mean for each point* (n = 6).

[2] Sasco Co., Omaha, Neb.
[3] Yellow Springs Instrument Co.

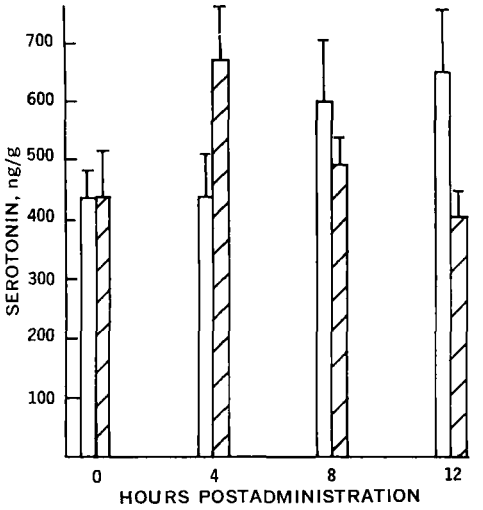

Figure 5—*Level of serotonin in the hypothalamus in control* (□) *and allylglycine-treated* (*intraperitoneally*) (▨) *groups. Brackets denote the standard error of the mean for each point* (n = 6).

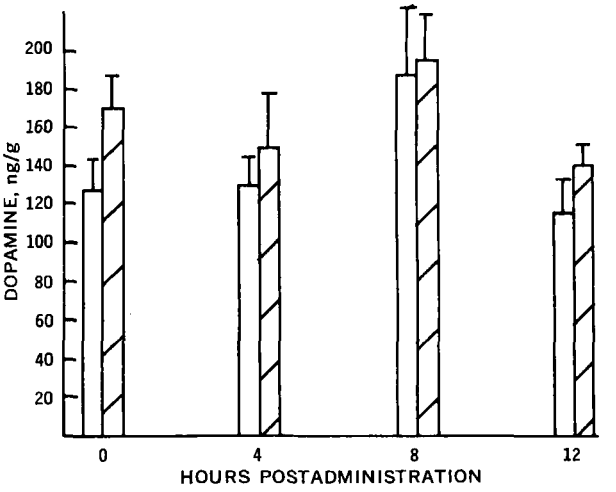

Figure 6—*Level of dopamine in the hypothalamus in control* (□) *and allylglycine-treated* (*intraperitoneally*) (▨) *groups. Brackets denote the standard error of the mean for each point* (n = 6).

To determine the central or peripheral sites affected by allylglycine in causing a temperature alteration, decapitated rats were used following the procedure of Weis (12). Rats were anesthetized with pentobarbital in a dose of 75 mg/kg ip. The trachea was cannulated and artificial respiration was initiated. The jugular veins and carotid arteries were ligated. The neck was clamped with a powerful hemostat to compress all ancillary vessels, and the head was cut off. The animals then were allowed to stabilize for 2 hr. Intraperitoneal injections of 0.9% saline or allylglycine were administered, and rectal temperature was monitored for 4 hr as previously described.

Cannulas were implanted into the lateral ventricles of anesthetized rats for the intraventricular administration of allylglycine. A 26-gauge needle, cut to proper length with a blunt end, was implanted stereotaxically into the ventricle at a point 2.5 mm lateral and 1 mm posterior to the bregma and secured to the skull with dental cement. A 30-gauge needle served as an injection cannula and occluding stylet. Then 1 week after the cannulas were implanted, 10 μl of either 0.9% saline or 0.2 *M* allylglycine was injected over 2 min and rectal temperature was monitored as previously described. Methylene blue was injected following the experiment for confirmation of the injection site.

Following the recording of rectal temperatures, 12 rats (six control and six allylglycine treated) were sacrificed by decapitation at 0, 4, 8, and 12 hr postadministration. The hypothalamus was removed following the procedure of Glowinski and Iversen (13), immersed in liquid nitrogen, and weighed. The tissue samples were wrapped in aluminum foil and stored at −20°. A method of solvent extraction coupled with alumina adsorption (14) was utilized for the assay of the monoamines. The fluorometric enzyme-coupled technique of Graham and Aprison (15) was used for γ-aminobutyric acid assay.

RESULTS

The intraperitoneal administration of allylglycine at 90 mg/kg caused a significant ($p < 0.05$) decrease in rectal temperature, $\Delta T = -3.1$ and $-1.1°$, when compared to the control group at 4 and 8 hr, respectively, postadministration (Fig. 1). A small, but insignificant, increase in rectal temperature was noted at 12 hr in the allylglycine-treated group when compared to the control.

At the dose of 90 mg/kg, compared to the 150-mg/kg dose used by Alberici *et al.* (10), no convulsions were seen and only three of 24 animals showed increased activity postadministration. All animals survived the 12-hr test period.

Intraperitoneal administration of allylglycine to the decapitated rat did not significantly decrease rectal temperature (Fig. 2). No significant change was noted at 1, 2, 3, and 4 hr after administration of either allylglycine or 0.9% saline.

Intraventricular administration of 10 μl of 0.2 *M* allylglycine resulted in a rapid decrease in temperature, with a significant ($p < 0.05$) decrease after 1 hr that lasted through 4 hr postadministration when compared to controls (Fig. 3). No significant temperature changes were noted in the control animals.

The data from the monoamine assay of the hypothalamic tissue show that allylglycine administration, 90 mg/kg ip, does not result in any significant changes in the levels of norepinephrine (Fig. 4), serotonin (Fig. 5), or dopamine (Fig. 6) at 4, 8, or 12 hr postadministration. However, a significant ($p < 0.05$) decrease in the hypothalamic γ-aminobutyric acid level does occur at 4, 8, and 12 hr postadministration (Fig. 7) when compared to control animals.

DISCUSSION

The absence of a significant decrease in rectal temperature in the decapitated animals treated with allylglycine suggests that this agent is causing its hypothermic action by acting at central thermoregulatory sites. With access to these sites eliminated by decapitation, any influence of the central thermoregulatory centers is removed, so the possibility that allylglycine causes hypothermia by affecting purely peripheral thermoregulatory mechanisms can be ruled out. This supposition of a purely central role in allylglycine

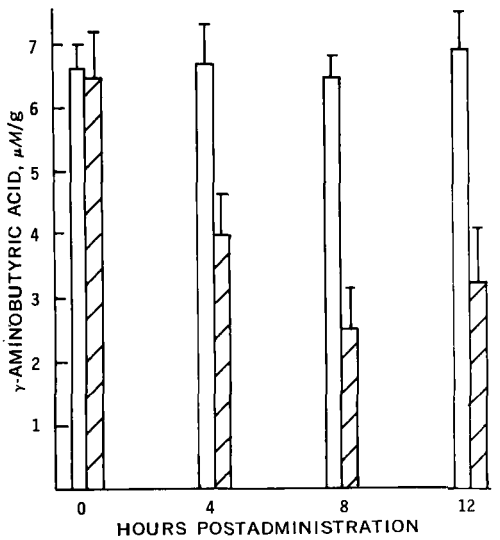

Figure 7—*Level of γ-aminobutyric acid in the hypothalamus in control* (□) *and allylglycine-treated* (*intraperitoneally*) (▨) *groups. Brackets denote the standard error of the mean for each point* (n = 6).

hypothermia is strengthened by the rapid onset of decreased temperature noted in intraventricularly injected rats (1 hr) as compared to intraperitoneally injected ones (4 hr).

The current theory of thermoregulatory control by the delicate balance of release of the two opposing monoamines, norepinephrine and serotonin, from the hypothalamus was mentioned previously. The results of this study indicate that no significant changes in the hypothalamic levels of norepinephrine or serotonin occurred at any time point after allylglycine and suggest that allylglycine affects monoamine turnover but causes no changes in content or that alteration of central γ-aminobutyric acid content produces this hypothermia. However, studies have shown that it is difficult to correlate a change in body temperature with a change in the hypothalamic levels of the putative neurotransmitters—*viz.*, norepinephrine and serotonin (16, 17). Here again, it may not be possible to propose a direct correlation between the temperature response seen and the hypothalamic tissue levels of the monoamines at the chosen time points of 4, 8, and 12 hr postadministration of allylglycine.

The studies of Alberici *et al.* (10) indicated that at 2–2.5 hr after administration of allylglycine, ultrastructural changes in the nerve endings of the cerebral cortex occurred, resulting in a reduction in the number of synaptic vesicles. Such alterations affected Fraction D of the glutamic acid decarboxylase-rich (nonaminergic) nerve endings obtained through subfractionation on a sucrose gradient (18). The fact that glutamic acid decarboxylase is inhibited *in vivo* and *in vitro* by allylglycine suggests that the decrease in γ-aminobutyric acid induced by this agent is directly related to the effect on the synthesizing enzyme, glutamic acid decarboxylase.

Allylglycine would also be expected to inhibit the high activity of this enzyme in the hypothalamus at the dose of 90 mg/kg as compared to the 150-mg/kg dose used by Alberici *et al.* (10), since significant ($p < 0.05$) decreases in the γ-aminobutyric acid content of the hypothalamic tissue were found in this study (Fig. 7). The greatest decrease in γ-aminobutyric acid, as compared to controls, was observed at 8 hr after allylglycine administration, followed by the 12- and 4-hr time points. Alberici *et al.* (10) completed their studies at 2–2.5 hr after allylglycine administration, since this was the time point noted for the greatest convulsive activity at the 150-mg/kg dosage. The time course of action of these effects on neuronal integrity of γ-aminobutyric acid neurons and glutamic acid decarboxylase is consistent with the time course of the allylglycine-induced hypothermia.

The greatest decrease in rectal temperature occurred 4 hr after allylglycine administration. The temperature of the animals then began to increase; at 12 hr, the allylglycine-treated group had an increased body temperature when compared to the control group. Thus, maximal allylglycine-induced hypothermia (at 4 hr) does not correlate with the greatest decrease in hypothalamic γ-aminobutyric acid levels (8 hr), but this maximum decrease at 4 hr may be caused by the ultrastructural changes in the nerve endings that could occur prior to the change noted at 4 hr. If the terminals are damaged and there is a reduction in the number of synaptic vesicles that store γ-aminobutyric acid, the initial release of γ-aminobutyric acid from the terminals may cause a postsynaptic stimulation of mechanisms that results in an inhibition of thermogenesis and/or an increased dissipation of body heat. The continued inhibition of glutamic acid decarboxylase by allylglycine can explain the further decrease in γ-aminobutyric acid levels at 8 and 12 hr. However, it would appear that after the 4-hr time point, a mechanism is brought into action to conserve the body heat and results in an increased body temperature, which results in the overcompensation noted at 12 hr after allylglycine administration.

In summary, the results indicate an involvement of γ-aminobutyric acid in mammalian thermoregulatory control. When synthesis of this putative neurotransmitter is impaired, a concomitant decrease in body temperature is noted. The mechanistic role of γ-aminobutyric acid in this system remains to be elucidated, although initial studies (19) point toward a modulatory role for this putative neurotransmitter in thermoregulation.

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