Biochemical Pharmacology, Vol. 40, No. 12, pp. 2637–2640, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00
Pergamon Press plc

PYRETIC ACTION OF LOW DOSES OF γ-HYDROXYBUTYRATE IN RATS

ELAINE E. KAUFMAN,* LINDA J. PORRINO and THOMAS NELSON

Laboratory of Cerebral Metabolism, National Institute of Mental Health, United States Public Health Service, Department of Health and Human Services, Bethesda, MD 20892, U.S.A.

(*Received* 29 *January* 1990; *accepted* 25 *June* 1990)

Abstract—γ-Hydroxybutyrate (GHB) has been found to have a biphasic effect on body temperature with increased body temperature after low doses (5–10 mg/kg) and decreased body temperature after high doses (300–500 mg/kg). Brain levels of GHB between 30 and 60 min post-injection of GHB were not altered by the low doses (5–10 mg/kg), although a dose of 200 mg/kg produced a large increase in the brain concentration.

It has been known for some time that the putative neurotransmitter [1], γ-hydroxybutyrate (GHB), when administered to animals in large pharmacological doses, produces a sharp drop in body temperature [2], marked behavioral and electroencephalographic (EEG) changes [3], a profound decrease in cerebral energy metabolism [4], and an increase in striatal dopamine levels together with a decreased impulse flow in dopaminergic neurons [5]. The characteristic pharmacological effects of GHB, together with the findings of Maitre and Mandel [1] which demonstrate specific binding sites for GHB in brain, have led to speculation that endogenous GHB may play a role either as a neuromodulator or possibly as a neurotransmitter in the central nervous system. However, the difference between the brain levels resulting from the pharmacological doses used to elicit the pharmacological effects described above and the endogenous level of GHB in the brain ranges from 500- to 1000-fold [6]. This discrepancy raises difficulties in proposing a role for endogenous GHB as a neuromodulator in the central nervous system.

In this study we have investigated one of the pharmacological actions of GHB, namely its effect on body temperature. It is known that in the rat high doses of GHB or γ-butyrolactone (GBL) lower body temperature [2]. Nothing, however, is known about the effects of low doses of GHB. The primary purpose of this study, therefore, was to examine the effects of GHB on body temperature in doses which more closely approximate its endogenous level.

MATERIALS AND METHODS

Adult male Sprague–Dawley rats weighing 300–400 g were obtained from Taconic Farms (Germantown, NY). The animals were housed in group cages under controlled temperature and humidity with a 12 hr light–dark cycle (lights on at 7:00 a.m.). Food and water were available *ad lib*. Sodium γ-hydroxybutyrate was purchased from the Sigma Chemical Co. (St. Louis, MO). Sodium chloride, 0.9% for injection, U.S.P. was obtained from Abbot Laboratories (Chicago, IL).

The experiments were carried out in a quiet room with ambient temperatures between 20.5 and 23°. Experiments were always started between 1:00 p.m. and 3:00 p.m. to avoid diurnal effects. Rectal temperatures were measured with a tele-thermometer (model 43KTH, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). The thin flexible probe (YSI-402) was inserted approximately 10 cm into the rectum. An initial temperature reading was taken before the intraperitoneal injection of either GHB or saline; readings were taken every 15 min thereafter for 75 min. To minimize the temperature elevation due to stress the rats were introduced to the procedure by subjecting them to temperature measurements, saline injection, and weighing on the day before the experiment.

RESULTS

The change in core body temperature from zero time to 60 min post-injection as a function of the dose of GHB is shown in Fig. 1. Low doses of GHB, 5 and 10 mg/kg, produced statistically significant increases in body temperature, whereas high doses of GHB, 300–500 mg/kg, produced significant decreases in body temperature.

Figure 2 compares the time–course of the core body temperature of rats following injections of either saline, a low dose (10 mg/kg) of GHB, or a high dose (500 mg/kg) of GHB. Both the saline-injected control rats and the rats administered 10 mg/kg GHB developed a persistent and relatively constant temperature elevation above that which had been measured before injections; however, the temperature rise associated with the low dose of GHB was significantly higher than that of the saline-injected control rats between 30 and 75 min. The high dose of GHB resulted in a core temperature that continued to decline until it reached a minimum at 60 min following the injection. Figure 3 displays the time–course of the changes in the core body temperatures in control rats administered an intraperitoneal injection of physiological saline and rats

* Correspondence: Dr. Elaine Kaufman, Laboratory of Cerebral Metabolism, National Institute of Mental Health, Building 36, Room 1A-05, Bethesda, MD 20892.

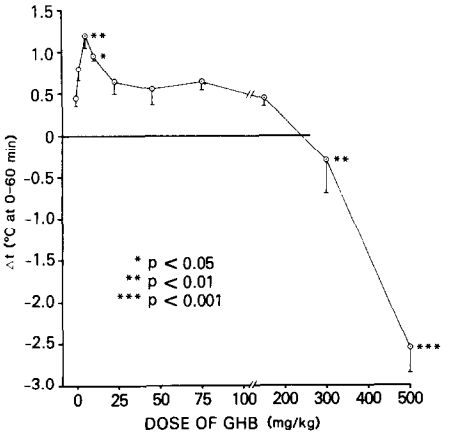

Fig. 1. Effect of various doses of GHB on core body temperature. Each point is the mean ± SE of the change in core temperature from 0 to 60 min. The number of rats (N) for the various points are: saline (23); 1 mg GHB/kg (14); 5 mg GHB/kg (9); 10 mg GHB/kg (13); 20 mg GHB/kg (6); 40 mg GHB/kg (6); 75, 150, 300 and 500 mg GHB/kg (5) each. A significant change in core temperature between experimental and saline control rats is indicated as (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ (one-way ANOVA followed by Dunnett's test for each dose compared to control).

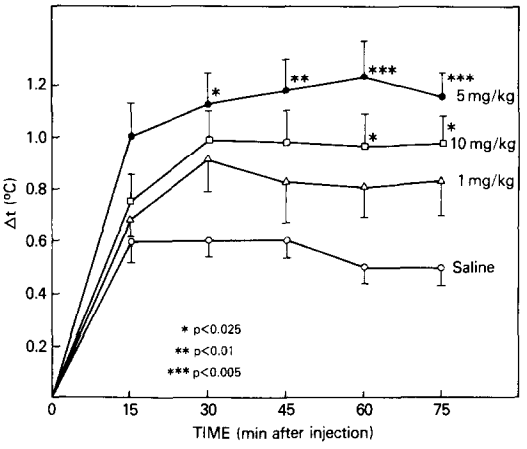

Fig. 3. Time–course of the changes in body temperature following injections of saline, 1 mg/kg of GHB. 5 mg/kg of GHB and 10 mg/kg of GHB. Each point is the mean ± SE of the change in core temperature from 0 to 15, 30, 45, 60 or 75 min. The number of animals used for the various concentrations of GHB is the same as indicated in the legend of Fig. 1. At each time point, a significant change in core temperature from that observed in the saline-treated animals is indicated by: (*) $P < 0.025$, (**) $P < 0.01$ and (***) $P < 0.005$ (Student's t-test).

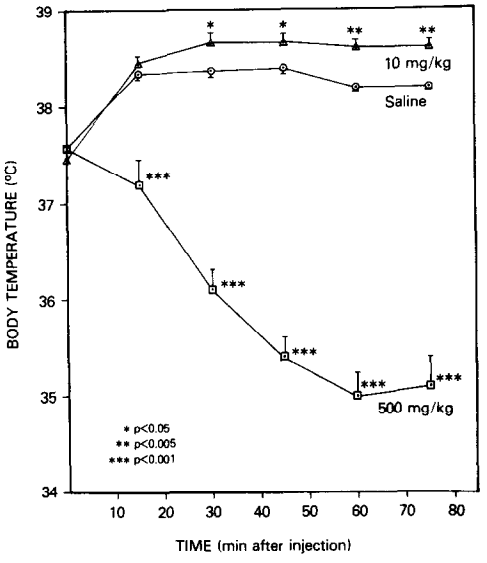

Fig. 2. Time–course of the core body temperature following injections of saline, a low dose (10 mg/kg), or a high dose (500 mg/kg) of GHB. Each point is the means ± SE of the core temperature at time (t). The numbers of animals used for the various concentrations are the same as indicated in the legend of Fig. 1. A significant change in core temperature in the experimental animals compared to the control animals is indicated by (*) $P < 0.05$, (**) $P < 0.005$ and (***) $P < 0.001$ (Student's t-test). Comparisons were made between the core temperature change in the experimental and control animals for each time point.

given 1, 5, and 10 mg/kg GHB. Each of these groups developed elevated temperatures which persisted for at least 75 min, but only the 5 and 10 mg/kg doses gave temperatures significantly higher than those of the controls. The 1 mg/kg GHB dose produced apparent temperature increases which exceeded those of the controls, but the effects seen at this dose were too variable to be statistically significant.

To determine the extent to which a given dose of GHB altered the brain level of this compound, animals were killed 60 min after an intraperitoneal injection of GHB, and the whole brains were assayed for GHB as described by Nelson *et al.* [7]. Doses of up to 20 mg/kg of GHB produced no detectable effect on the brain level of GHB (Fig. 4), although doses of 5 and 10 mg/kg produced significant elevations in body temperature. A dose of 75 mg/kg produced a small increase in the concentration of GHB in the brain, whereas a dose of 200 mg/kg increased the brain GHB concentration to levels approximately 40-fold higher than either the normal brain concentration of GHB or that found with doses of 0–20 mg/kg. In a separate experiment (data not shown), the effect of a dose of 5 mg/kg on brain levels of GHB was determined in animals 30 min after the intraperitoneal injection. No effect of 5 mg/kg of GHB on brain GHB levels could be seen at this earlier time point.

DISCUSSION

One of the first reports on the effect of GHB on body temperature was that of Laborit *et al.* [2] who demonstrated that high doses of GHB (500 mg/kg) can depress body temperature. The results of our experiments with high doses of GHB are in accord with those of Laborit *et al.* [2] but not with those of Borbély and Huston [8] who found a triphasic effect with 400 mg/kg of GBL. Significant differences exist, however, between our experimental conditions and

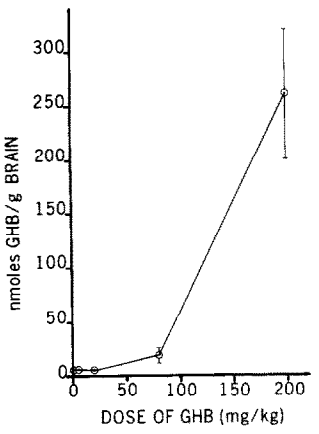

Fig. 4. Brain levels of GHB produced by various doses of GHB. The concentration of GHB in the whole brain of rats was determined 60 min after an i.p. dose of either saline (N = 4) or 5, 20, 80, or 200 mg/kg of GHB (N = 3). Each point is the mean ± SE of either 3 or 4 determinations. The SE for the determinations in animals that received saline (0), 5 and 20 mg/kg of GHB (not shown in the figure) were 0.45, 0.41 and 0.35 nmol/g brain respectively.

those of Borbély and Huston: (1) the ambient temperature at which their experiments were carried out was 30°, significantly higher than the ambient temperature was for this study (20.5 to 23.0°), (2) GBL rather than GHB was used, and (3) finally, Borbély and Huston used a "counterbalanced design with intervals of at least two days between experiments." The animals used by Borbély and Huston, therefore, had been chronically exposed to GBL. We observed that with repeated administration of GHB over a period of several days the changes in core temperature were altered from those observed in naïve animals (unpublished results).

The focus of this work has been on the effect of very low doses of GHB on body temperature. One of the problems with the hypothesis that GHB plays a role in modulating the physiological activity of central dopaminergic neurons [6] or functions as a neuromodulator or neurotransmitter is the discrepancy between the normal brain levels of GHB and the 500- to 1000-fold higher brain levels observed following commonly used pharmacological doses. The elevation of core temperature in the rat produced by the 5 and 10 mg/kg doses of GHB provides the first indication of a pharmacological effect of this compound at very low doses. The lowest subanesthetic doses of GHB in rats and mice previously found to produce pharmacological effects were those reported by Roth *et al.* [6] who found an effect on 3,4-dihydroxyphenylacetic acid (DOPAC) levels at 75 mg/kg i.v. in the rat, and by Krsiak *et al.* [9], who found an effect on passive defensive behavior in mice at 50 mg/kg. The effects of low doses of GHB have been studied in the human by Takahara *et al.* [10] who reported a stimulatory effect on the release of growth hormone and prolactin in humans with an intravenous dose of 2.5 g (approximately 35 mg/kg for a 70 kg man), and by Grove-White and Kelman [11, 12] who found a change in critical flicker frequency and short-term memory at an intravenous dose of 10 mg/kg in humans. The intraperitoneal route of administration used in our study would give lower blood levels of GHB than those that occurred in both the human and animal studies cited above. The low doses of GHB required to elicit a response in the human studies may be attributable to both the i.v. route of administration and to the lower metabolic rate of humans as compared to that of rats and mice.

The high doses (200–1000 mg/kg) which have been used previously have led to large increases in the level of GHB in the brain; however, the low doses (5–10 mg/kg) studied here produced no detectable change in whole brain levels of GHB at 30–60 min after the injection of GHB, the time at which the maximum effect on core temperature was observed (Fig. 4). Thus, hyperthermia appears to occur when there have been either very small or possibly region-specific changes in GHB content which are not reflected in measurements made on whole brain. Hypothermia, however, appears to be correlated with large increases in the GHB concentration of the brain. The distinctly different responses to the high and low doses of GHB suggest that an additional mechanism of action comes into play at the high dosage level.

Recently, similarities between some of the neuropharmacological effects of GHB and those of opiates and opiate peptides have been reported [13]. The significance of this similarity is supported by the finding of Snead and Bearden [13] that the specific opiate antagonists, naloxone and naltrexone, attenuated or abolish the EEG and behavioral changes as well as the increase in striatal dopamine levels produced by the administration of GBL. Moreover, Crosby *et al.* [14] have demonstrated that naloxone pretreatment reverses the GHB-induced depression in cerebral glucose utilization in 10 of 38 cerebral structures examined. In light of these findings it is interesting to note the marked similarity between the effects of high and low doses of morphine [15, 16] and of GHB (Fig. 1) on body temperature. Both compounds produce hyperthermia at low doses and hypothermia at higher doses. Hermann [16] first reported that doses of 1–20 mg/kg morphine produce moderate hyperthermia in the rat, whereas doses of 40–90 mg/kg produce hypothermia. Holaday *et al.* [17] have also reported that hyperthermia is seen following administration of low doses of β-endorphins to rats, whereas hypothermia is seen following administration of high doses of β-endorphin.

REFERENCES

1. Maitre M and Mandel P, Neurobiology, Propriétés permettant d'attribuer au γ-hydroxybutyrate la qualité de neurotransmetteur du système nerveux central. *C R Acad Sci [III]* **298**: 341–345, 1984.
2. Laborit H, Jouany JM, Gerard J and Fabiani F, Résumé d'une étude expérimentale et clinique sur un substrat métabolique a action central inhibitrice le 4-hydroxybutyrate de Na. *Presse Med* **68**: 1867–1869, 1960.
3. Snead OC III, Gamma hydroxybutyrate in the monkey. I: Electroencephalographic, behavioral, and pharmacokinetic studies. *Neurology* **28**: 636–642, 1978.

4. Wolfson LI, Sakurada O and Sokoloff L, Effects of γ-butyrolactone on local cerebral glucose utilization in the rat. *J Neurochem* **29**: 777–783, 1977.
5. Roth RH and Suhr Y, Mechanism of the γ-hydroxybutyrate-induced increase in brain dopamine and its relationship to "sleep." *Biochem Pharmacol* **19**: 3001–3012, 1970.
6. Roth RH, Doherty JD and Walters JR, Gamma-hydroxybutyrate: A role in the regulation of central dopaminergic neurons. *Brain Res* **189**: 556–560, 1980.
7. Nelson T, Kaufman E, Kline J and Sokoloff L, The extraneural distribution of γ-hydroxybutyrate. *J Neurochem* **37**: 1345–1348, 1981.
8. Borbély AA and Huston JP, γ-Butyrolactone: An anesthetic with hyperthermic action in the rat. *Experientia* **28**: 1455, 1972.
9. Krsiak M, Novakova D, Paclt I and Ustrovskaya RU, Effect of sodium hydroxybutyrate on behavior of mice after prolonged isolation. *Bull Exp Biol Med* **77**: 288–291, 1974.
10. Takahara J, Yunoki S, Yakushiji W, Yamauchi J, Yamane Y and Ofuji T, Stimulatory effects of gamma-hydroxybutyric acid on growth hormone and prolactin release in humans. *J Clin Endocrinol Metab* **44**: 1014–1017, 1977.
11. Grove-White IG and Kelman GR, Critical flicker frequency after small doses of methohexitone, diazepam and sodium 4-hydroxybutyrate. *Br J Anaesth* **43**: 110–112, 1971.
12. Grove-White IG and Kelman GR, Effect of methohexitone, diazepam and sodium 4-hydroxybutyrate on short-term memory. *Br J Anaesth* **43**: 113–116, 1971.
13. Snead OC III and Bearden LJ, Naloxone overcomes the dopaminergic, EEG, and behavioral effects of γ-hydroxybutyrate. *Neurology* **30**: 832–838, 1980.
14. Crosby G, Ito M, Kaufman E, Nelson T and Sokoloff L, Naloxone pretreatment alters the local cerebral metabolic effect of γ-hydroxybutyrate in rats. *Brain Res* **275**: 194–197, 1983.
15. Gunne LM, The temperature response in rats during acute and chronic morphine administration. A study of morphine tolerance. *Arch Int Pharmacodyn* **129**: 416–428, 1960.
16. Hermann JB, The pyretic action on rats of small doses of morphine. *J Pharmacol Exp Ther* **76**: 309–315, 1942.
17. Holaday JW, Wei E, Loh HH and Li CH, Endorphins may function in heat adaption. *Proc Natl Acad Sci USA* **75**: 2923–2927, 1978.