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Gammahydroxybutyric Acid: Central Biochemical and Behavioral Effects in Neonatal Rats

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HEDNER, T., J. HEDNER, K. IVERSEN, P. WESSBERG AND P. LUNDBORG. *Gammahydroxybutyric acid: Central biochemical and behavioral effects in neonatal rats.* PHARMACOL BIOCHEM BEHAV 23(2) 185–189, 1985.—Administration of gammahydroxybutyric acid (GHBA) to 4 days old animals caused a dose dependent decrease in locomotor activity. GHBA also induced a marked hypoventilation, irregular breathing and finally apnea, while heart rate was slightly increased. Changes in monoamine neurotransmitter turnover indicated an inhibition of dopamine (DA) neurotransmission. It is concluded that GHBA mechanisms in the neonatal rat brain are biochemically as well as functionally mature at an early age and that the effects on locomotor activity and respiratory regulation at least partly may involve interactions with central DA neurotransmission.

Neonate CNS GHBA

GAMMAHYDROXYBUTYRIC acid (GHBA) is an endogenous brain constituent with potent neurophysiological and neuropharmacological properties [32]. Its actions have previously largely been associated with gammaaminobutyric acid (GABA) as GHBA may be formed by metabolism of GABA in the brain [14,31]. However, recent data indicate that GHBA may be formed from important sources other than GABA such as polyamines [11] and the developmental time table [33] is different from GABA. This suggests that GHBA may play a biologically significant role as a neuroactive agent with independent actions in neurotransmission or neuromodulation.

The ontogenetic development of endogenous GHBA concentrations in the brain are well described in several species [33]. Thus, e.g., in the rat, concentrations are high during early development and decrease significantly during postnatal development. Previous investigations from our laboratory [21,28] have furthermore indicated that central GHBA mechanisms are also functionally mature during early age.

Thus, if neuronal pathways for GHBA exist in the brain, they demonstrate a high degree of maturity during early age, which may infer a role in physiological or pathophysiological conditions during the neonatal period. In order to further investigate the functional neuromodulatory role of GHBA during early age, we have studied the effects on monoamine neurotransmission, as well as the effects on heart rate, respiration and motor behaviour in 4 days old rats.

METHOD

Pregnant Sprague-Dawley rats (Anticimex, Stockholm) were obtained at the 16–17 day of gestation, and housed in the department under regulated dark-light conditions (light period 6 a.m.–8 p.m.). Parturition occurred at 22±1 days of gestation and the time of delivery was noted within 12 hr. Experiments, biochemical as well as behavioural, were performed on the rat pups at 4 days of postnatal age.

Locomotor Activity

In order to measure locomotor activity, the neonatal rats were placed in a transparent cage (195×300 mm, height 245 mm), put in a ventilated and sound proof box with a one-way mirror for observation. Locomotor activity was measured using the "M/P 40 Fc Electron Motility Meter" (Motron Products, Stockholm) [29]. This instrument is equipped with 40 photoconductors (5 rows of 8, centre-centre distance 40 mm) and covered by a translucent floor. The transparent cage was placed over the photoconductors, and on top of the cage a black-painted "chimney" was mounted in order to prevent light scattering. The light source was a 12 V car bulb which was placed in the roof of the chamber. Every tenth interruption of the photocell beam was recorded as one count. The recordings were made for 15 min periods. After a 15 min control period, GHBA, 750 mg/kg, was injected SC, and locomotor activity was recorded for 90 min. In separate experiments, accumulated locomotor activity (60 min) was

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measured 30 min after increasing doses of GHBA (75–1500 mg/kg). Control animals received saline. In the experiments, 4 animals were run together and each animal was used only once.

Heart Rate and Respiratory Frequency

Measurements were performed before GHBA (750 mg/kg SC) or saline injection and at every 10 min interval for one half hour after the administration. Heart rate (HR) was calculated from ECG recordings applying electrodes on both front and the right back limbs. Respiratory frequency was obtained from an external inflated rubber device applied around the thoracic cage [6]. The volume changes were measured by a low pressure transducer (Statham PT 5A) connected to a Grass polygraph.

Biochemistry

GHBA (750 mg/kg) or saline was administered subcutaneously 90 min before sacrifice. Animals whose brains were analysed for tyrosine, DOPA, tryptophan and 5-HTP were given NSD 1015 (3-hydroxy-benzylhydrazine HCl), 100 mg/kg SC, 30 min before decapitation. NSD 1015 is a potent inhibitor of aromatic L-amino acid decarboxylase in the brain and causes increases in the concentrations of the monoamine precursors DOPA and 5-HTP. The measurement of these intermediates in the brain is thus a good method to estimate the *in vivo* activity of the rate limiting enzymes, tyrosine hydroxylase and tryptophan hydroxylase, respectively [10]. Animals intended for analysis of DA, NA, 5-HT and 5-HIAA did not receive NSD 1015 injections.

The rats were killed by decapitation and the whole brain was dissected out and immediately frozen on solid CO_2. Three brains were pooled per sample for the biochemical analyses. All brain samples were stored at −70°C, in no case for more than three months. After thawing, the brain samples were homogenized in 10 ml 0.4 perchloric acid containing 5 mg sodium metadisulfite ($Na_2 S_2 O_5$) and 20 mg EDTA. The extracts were purified on a strong cation exchange column (Dowex 50-X-4) and analysed spectrofluorometrically for tyrosine, DOPA, DA, NA, tryptophan, 5-HTP, 5-HT and 5-HIAA according to previously described techniques [3, 4, 5, 7, 9, 12, 24, 34].

Drugs Used

The following drugs were used in the study: GHBA (sodium form, Sigma Chemical Co., St. Louis, MO) (NSD 1015 synthesized in this laboratory).

Statistical Methods

Conventional methods were used for the calculations of means±s.e.m. Tests of significance were conducted using one way analysis of variance or Student's *t*-test. *p*-Values larger than 0.05 were considered non significant.

RESULTS

After a single injection of GHBA (750 mg/kg) there was a progressive decline in locomotor activity reaching its lowest levels after 45 min (Fig. 1a). After this time, spontaneous locomotor activity was almost absent in the GHBA treated group. Administration of GHBA (75–1500 mg/kg SC) to the neonatal rats, caused a dose-dependent reduction of locomotor activity (Fig. 1b).

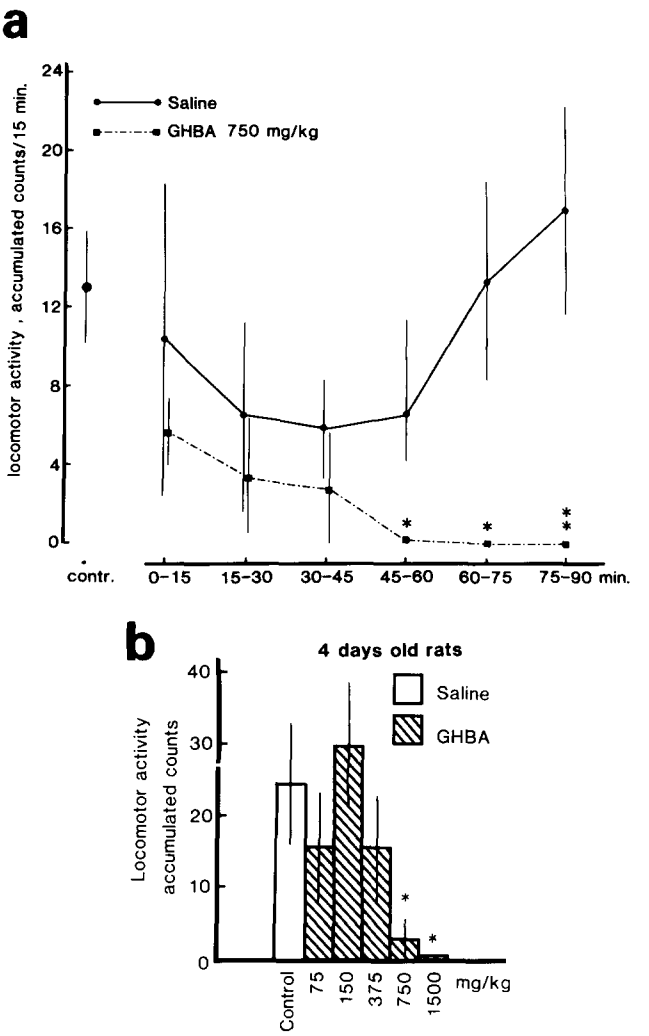

FIG. 1a and b. Locomotor activity in 4 days old rats (accumulated counts per 15 min) after a single injection of 750 mg/kg of GHBA (a) and accumulated locomotor activity (60 min) in rat neonates 30 min after SC injections of increasing doses of GHBA (75–1500 mg) (b). Controls received an equal volume of saline. Shown are means± s.e.m. of 7–8 neonates in each group. Statistical comparison with *t*-test. $*p<0.05$, $**p$x0.01.

Respiratory frequency (f) was already significantly decreased 10 min after GHBA administration (Fig. 2). After 30 min, f was approximately 10–15% of the normal value, and in some animals marked irregular breathing as well as apnea appeared (Fig. 3). At this time, 30–40 min after injection, most animals appeared cyanotic. No spontaneous breathing was seen after 40 min and therefore respiratory rate was not further followed. In contrast to this, HR increased significantly after GHBA administration (Table 1). Thus, 30 min after injection, HR was approximately 20% increased compared to the preinjection control.

After GHBA and NSD 1015 there was a significant approximately 50% increase in DOPA accumulation concomitant with an increase in whole brain tyrosine (Table 2). DA levels were also increased while NA tended to decrease, however not significantly. Whole brain 5-HTP accumulation was decreased in the neonatal animals after GHBA and NSD

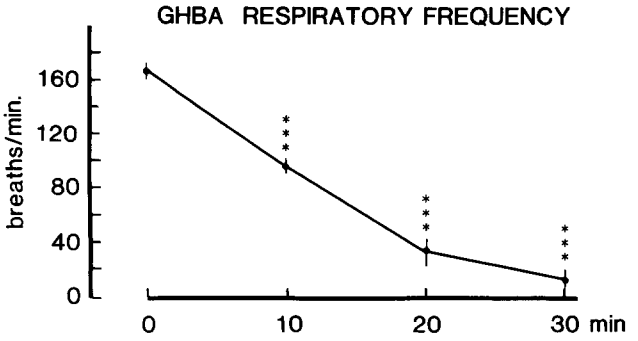

FIG. 2. Respiratory frequency in unanaesthetized 4 days old rats after a single injection of GHBA (750 mg/kg SC). Shown are means±s.e.m. of 6 animals. Statistical comparison to initial control (0 min) value by t-test. ***$p<0.001$.

TABLE 1

EFFECT OF GHBA ON HEART RATE IN 4 DAYS OLD RATS

Time after injection (min)	Heart rate (beats/min) GHBA (n=5)
0	361 ± 18.7
10	411 ± 10.0*
20	432 ± 14.5†
30	431 ± 14.9†

Shown are beats/min (means ± s.e.m.) before and at various times after administration of GHBA, 750 mg/kg SC. Statistics by one way analysis of variance followed by t-test. Significances indicated vs. preinjection (0 min) value. *$p<0.05$, †$p<0.01$.

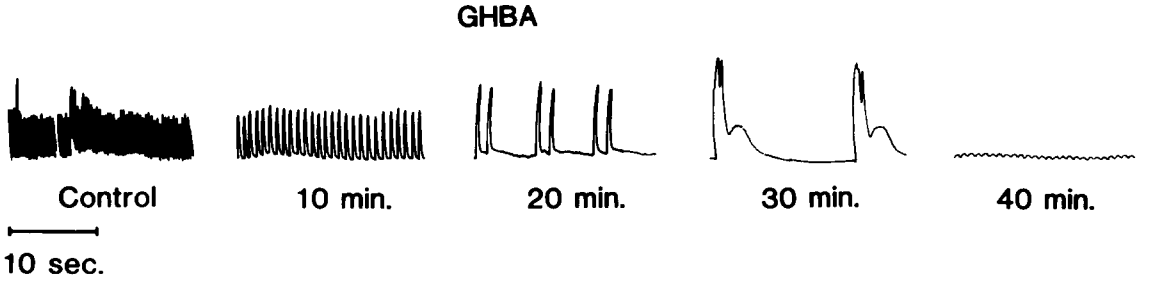

FIG. 3. Replica of an original representative respiratory recording from a 4 days old rat neonate after a SC injection of GHBA, 750 mg/kg SC. Inspiration upwards. Note irregular periodic breathing appearing 20 min after injection and apnea at 40 min.

1015, while tryptophan, 5-HT and 5-HIAA levels remained unaltered (Table 2). We chose to evaluate the biochemical parameters at a time when the locomotor effects were fully developed, i.e., 60 to 90 min after GHBA injection.

DISCUSSION

GHBA elicits profound behavioural effects when administered to adult rats. Thus after systemic administration, there is predominantly a reduction in locomotor activity [35], decrease in body temperature [26] and a respiratory depression [17]. However, little effect is seen on cardiovascular parameters, and blood pressure remains essentially unaltered [30], while HR may be unchanged or slightly increased [16,30].

In our investigation in neonatal rats, there were marked changes in locomotor activity indicating a high degree of functional maturity of GHBA mechanisms in the CNS during early postnatal age. The effects on locomotor activity were clearly dose dependent and at the highest doses marked reductions of spontaneous locomotor activity were seen. The anatomical basis for effects on locomotor activity is not known, but could be the result of, e.g., a direct effect due to, activation of postsynaptic receptors for GHBA [8] or an indirect effect due to inhibition of the nerve impulse flow in the DA nigro-striatal neuronal pathways [1,35].

Exogenous GHBA induced a profound hypoventilation in the unanaesthetized neonatal rat, which is in agreement with previous findings in anaesthetized adult animals [16]. This effect was not lethal, indicated by the observation that HR

TABLE 2

EFFECTS OF GHBA ON ENDOGENOUS WHOLE BRAIN MONOAMINE LEVELS IN 4 DAYS OLD RATS

	Control	GHBA (750 mg/kg)	p
Tyrosine (μg/g)	22.8 ± 1.62 (6)	34.2 ± 1.51 (8)	<0.001
DOPA (ng/g)	65 ± 7 (6)	102 ± 8 (9)	<0.001
Dopamine (ng/g)	136 ± 6 (4)	183 ± 12 (3)	<0.05
Noradrenaline (ng/g)	122 ± 12 (4)	87 ± 15 (3)	n.s.
Tryptophan (μg/g)	6.1 ± 0.56 (6)	5.7 ± 0.25 (4)	n.s.
5-HTP (ng/g)	49 ± 3 (7)	31 ± 5 (4)	<0.05
5-HT (ng/g)	58 ± 16 (5)	72 ± 15 (5)	n.s.
5-HIAA (ng/g)	95 ± 8 (5)	98 ± 8 (6)	n.s.

Shown are means ± s.e.m. GHBA treated animals were injected SC 90 min before sacrifice. Conrols received an equal volume of saline. At 30 min before decapitation, all animals analysed for tyrosine, DOPA, tryptophan and 5-HTP were given NSD 1015, 100 mg/kg SC. Statistical comparison by t-test.

was increased in the treated neonates. In fact, GHBA increases hypoxic survival in adult animals [2], when the agent is administered in the same dose range as used in this study. The beneficial effect of GHBA in this respect was due to a decrease in cerebral metabolic rate for oxygen [2]. Although not demonstrated, this mechanism is most probably also applicable for the neonatal animal as the newborn has an even higher capacity to survive oxygen deprivation [19,20]. The respiratory depressant effects induced by GHBA are most probably of CNS origin [16,17] although the potential for GHBA to inhibit respiration seems to be more pronounced when administered by the intraperitoneal [17] compared to the intracerebroventricular route [16]. The respiratory effects induced by GHBA also seem to differ qualitatively from those elicited by GABA or GABA receptor agonists like muscimol [16,17]. This may indicate that GHBA alters respiration through mechanisms which are independent of those affected by GABA as indicated by their different effects on respiration in neonatal animals [17]. This assumption may also be supported by the fact that the time tables for the development of GHBA [33] and GABA mechanisms [18] follow different profiles.

The effects of GHBA on the turnover of monoamine neurotransmitters indicated an interruption of the nerve impulse traffic in DA neurons as evidenced by the increased DA levels and DOPA accumulation in the brain. This is in agreement with previous results reported in both adult and developing animals [1, 13, 21, 28]. These effects on DA turnover are probably due to an inhibition of the nerve impulse flow of the DA nigrostriatal neuronal pathways [1,13]. There was, however, no effect on NA neurons, while 5-HT synthesis was slightly decreased. This latter finding may be a result of the hypoxia induced by GHBA, as oxygen deprivation causes similar changes in 5-HT synthesis [20]. Administration of GHBA to adult animals seems to increase 5-HT synthesis [22] and degradation [36] in agreement with the effects on catecholamine turnover. In neonatal animals, however, a decrease has been found after prolonged administration [22]. The effects elicited by GHBA on catecholamine turnover (increased catecholamine synthesis and increased DA levels) are in contrast unlike those seen after hypoxia (decreased catecholamine synthesis, unchanged endogenous levels of DA) [19]. It should also be noted that the biochemical as well as the behavioural effects elicited by GHBA were seen after relatively high doses. The fact that effects can be elicited does not necessarily mean that the GHBA system is operating under physiological conditions in the neonate animal. However, from data on distribution [11,33] and the existence of binding sites for GHBA in the brain [8], it is tempting to speculate that this is the case.

Summarizing our observations, it can be concluded that GHBA exhibits marked locomotor and respiratory effects in neonatal animals, which partly may be due to direct postsynaptic effects and partly due to inhibition of DA neurotransmission, as DA agonists stimulate both locomotor activity [23, 25, 35] as well as respiratory frequency [15,27]. However, most importantly, central GHBA mechanisms during early postnatal age demonstrate a high degree of biochemical as well as functional maturity.

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REFERENCES

1. Andén, N.-E. and G. Stock. Inhibitory effect of gammahydroxybutyric acid and gammaaminobutyric acid on the dopamine cells in the substantia nigra. *Naunyn Schmiedebergs Arch Pharmacol* **279:** 89–92, 1973
2. Artru, A. A., P. A. Steen and J. P. Michenfelder. γ-Hydroxybutyrate: Cerebral metabolic vascular and protective effects. *J Neurochem* **35:** 1114–1119, 1980.
3. Atack, C. V. The determination of dopamine by a modification of the dihydroxyindole fluorimetric assay. *Br J Pharmacol* **48:** 699–714, 1973.
4. Atack, C. and M. Lindqvist. Conjoint native and orthopthaldialdehyde condensate assays for the fluorometric determination of 5-hydroxyindoles in brain. *Naunyn Schmiedebergs Arch Pharmacol* **279:** 267–284, 1973.
5. Atack, C. and T. Magnusson. A procedure for the isolation of noradrenaline (together with adrenaline), dopamine, 5-hydroxytryptamine and histamine from the same tissue sample using a strongly acidic cation exchange resin. *Pharmacol Toxicol* **42:** 35–57, 1978.
6. Bass, N. H. and P. Lundborg. Respiratory depressant effects of morphine on the central nervous system of the infant rat. *Biol Neonate* **23:** 456–468, 1973.
7. Bédard, P., A. Carlsson and M. Lindqvist. Effect of a transverse cerebral hemisection on 5-hydroxytryptamine metabolism in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **272:** 1–15, 1972.
8. Benavides, J., J. F. Rumigny, J. J. Bourguignon, C. Cash, C. G. Wermuth, P. Mandel, G. Vincendon and M. Maitre. High affinity binding site for γ-hydroxybutyric acid in rat brain. *Life Sci* **30:** 953–961, 1982.
9. Bertler, A., A. Carlsson and E. Rosengren. A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta Physiol Scand* **44:** 273–292, 1958.
10. Carlsson, A., J. N. Davies, W. Kehr, M. Lindqvist and C. V. Atack. Simultaneous measurements of tyrosine and tryptophan hydroxylase activities in brain *in vitro* using an inhibitor of the aromatic amino acid decarboxylase. *Naunyn Schiedebergs Arch Pharmacol* **275:** 153–168, 1972.
11. Doherty, J. C., S. E. Hattox, O. C. Snead and R. H. Roth. Identification of endogenous γ-hydroxybutyrate in human and bovine brain and its regional distribution in human and guinea pig and rhesus monkey brain. *J Pharmacol Exp Ther* **207:** 130–139, 1978.
12. Duggan, D. E. and S. Undenfriend. The spectrophotofluorometric determination of tryptophan in plasma and of tryptophan and tyrosine in protein hydrolysates. *J Biol Chem* **223:** 313–319, 1956.
13. Gessa, G. L., L. Vargin, F. Crabai, G. C, Boero, F. Caboni and R. Cambra. Selective increase in brain dopamine induced by γ-hydroxybutyrate. *Life Sci* **5:** 1921–1930, 1966.
14. Gold, B. I and R. H. Roth. Kinetics of in vivo conversion of γ-[^{3}H]-amino-butyric acid to γ[^{3}H]-hydroxybutyric acid by rat brain. *J Neurochem* **28:** 1069–1073, 1977.
15. Hedner, J., T. Hedner, J. Jonason and D. Lundberg. Evidence for a dopamine interaction with the respiratory control system in the rat. *Eur J Pharmacol* **61:** 603–615, 1982.
16. Hedner, J., T. Hedner, J. Jonason and D. Lundberg. GABAergic mechanisms in central respiratory control in the anaesthetized rat. *Naunyn Schmiedbergs Arch Pharmacol* **317:** 315–320, 1981.

17. Hedner, T., J. Hedner, B. Bergman, K. Iversen and J. Jonason. Effects of GABA and some GABA analogues on respiratory regulation in the preterm rabbit. *Biol Neonate* **43:** 134–145, 1983.
18. Hedner, T., K. Iversen and P. Lundborg. Central GABA mechanisms during postnatal development in the rat. Neurochemical characteristics. *J Neural Transm*, **59:** 105–118, 1984.
19. Hedner, T. and P. Lundborg. Catecholamine metabolism in neonatal rat brain during asphyxia and recovery. *Acta Physiol Scand* **109:** 169–175, 1980.
20. Hedner, T. and P. Lundborg. Serotonin metabolism in neonatal rat brain during asphyxia and recovery. *Acta Physiol Scand* **109:** 163–168, 1980.
21. Hedner, T. and P. Lundborg. Effect of gammahydroxybutyric acid on catecholamine synthesis and utilization in the developing rat brain. *J Neural Transm* **54:** 19–28, 1982.
22. Hedner, T. and P. Lundborg. Effect of gammahydroxybutyric acid on serotonin synthesis, concentration and metabolism in the developing rat brain. *J Neural Transm* **57:** 39–48, 1983.
23. Jackson. D. M., N.-E. Andén and A. Dahlström. A functional effect of dopamine in the nucleus accumbens and in some other dopamine rich parts of the brain. *Psychopharmacologia* **45:** 139–149, 1975.
24. Kehr, W., A. Carlsson and M. Lindqvist. A method for the determination of 3,4-dihydroxyphenylalanine (DOPA) in brain. *Naunyn Schemidebergs Arch Pharmacol* **274:** 273–280, 1972.
25. Kellogg, C. and P. Lundborg. Ontogenic variations in responses to L-DOPA and monoamine receptor stimulating agents. *Psychopharmacologia* **23:** 187–200, 1972.
26. Lin, M. T., Y. F. Chern, H. S. Wang and A. Chandra. Effect of γ-hydroxybutyric acid on metabolic, respiratory and vasomotor activities and body temperature in rats. *J Pharmacol Exp Ther* **211:** 167–170, 1979.
27. Lundberg, D., G. R. Breese and R. A. Mueller. Dopaminergic interaction with the respiratory control system in the rat. *Eur J Pharmacol* **54:** 153–159, 1979.
28. Lundborg. P., T. Hedner and J. Engel. Catecholamine concentration in the developing rat brain after γ-hydroxybutyric acid. *J Neurochem* **35:** 425–429, 1980.
29. Modigh, K. Central and peripheral effects of 5-hydroxytryptophan on motor activity in mice. *Psychopharmacologia* **23:** 48–54, 1972.
30. Persson, B. GABA-ergic mechanisms in blood pressure control. A pharmacological analysis in the rat. *Acta Physiol Scand (Suppl)* **491:** 1–54, 1980.
31. Roth, R. H. and N. M. Giarman. Conversion in vivo of γ-aminobutyric to γ-hydroxybutyric acid in the rat. *Biochem Pharmacol* **18:** 247–250, 1969.
32. Snead, O. C. Minireview. Gamma hydroxybutyrate. *Life Sci* **20:** 1935–1944, 1977.
33. Snead, O. C. and B. J. Morley. Ontogeny of γ-hydroxybutyric acid. I. Regional concentration in the developing rat, monkey and human brain. *Dev Brain Res* **1:** 579–589, 1981.
34. Waalkes, T. P. and S. Udenfriend. A fluorometric method for the estimation of tyrosine in plasma and tissues. *J Lab Clin Med* **50:** 733–736, 1957.
35. Wachtel, H. and N.-E. Andén. Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms. *Naunyn Schmiedebergs Arch Pharmacol* **302:** 133–139, 1978.
36. Waldmeier, P. C. and B. Fehr. Effects of baclofen and γ-hydroxybutyrate on rat striatal and mesolimbic 5-HT metabolism. *Eur J Pharmacol* **49:** 177–184, 1984.