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Effect of γ-Hydroxybutyrate on Central Dopamine Release *In Vivo*

A MICRODIALYSIS STUDY IN AWAKE AND ANESTHETIZED ANIMALS

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ABSTRACT. γ-Hydroxybutyrate (GHB) is generally considered to be an inhibitor of striatal dopamine (DA) release. However, a number of recent reports and at least one major review suggest that GHB enhances rather than inhibits striatal DA release. To examine this discrepancy, the effect of GHB on striatal DA release was monitored for 2 hr by microdialysis in awake and urethane-anesthetized rats. GHB (500 mg/kg, i.p.) significantly inhibited striatal DA release in conscious animals. However, anesthetic pretreatment completely abolished the inhibitory effect of GHB on DA release. In urethane-anesthetized animals, intraperitoneal injections of GHB resulted in a dialysis DA output that was the same as basal and saline control levels for all but the last three intervals where DA release was elevated slightly. In contrast to the intraperitoneal route, subcutaneous injections of GHB in anesthetized animals produced significant elevations of DA release above baseline levels. The increases ranged from 125 to 133% of basal levels. These results indicate that while GHB enhances striatal DA release in anesthetized animals, it inhibits rather than enhances this release in awake animals. This would explain why GHB induces an inhibition of DA-release-dependent behaviors rather than an enhancement. The results also indicate that the route of GHB administration influences its effects on striatal DA release, at least in anesthetized animals. *Copyright © 1996 Elsevier Science Inc.*, BIOCHEM PHARMACOL 53;1:103–110, 1997.

KEY WORDS. hydroxybutyrate; *in vivo;* microdialysis; striatal dopamine release; conscious rat; anesthetized rat

GHB is a short-chain 4-carbon fatty acid found in the brain of mammals 33 years ago [1]. Seven years later, Roth and Suhr [2] presented evidence that GHB serves as a relatively specific endogenous regulator of central DA neurons. Largely as a result of this and similar studies by Roth and co-workers in the early to middle 1970s, the compound became widely known as an inhibitor of central DA release. It has continued to be so regarded by virtue of its ability to block the firing of central DA neurons.

On the other hand, five compelling studies and a major review suggest that GHB exerts an excitatory effect on DA release. The first of these studies demonstrated an excitatory effect of GHB on central DA release using a push-pull cannula technique in the cat caudate *in vivo* following the peripheral administration of GHB [3]. More recently, Maitre *et al.* [4] observed a marked stimulation of rat striatal DA *in vivo*, using microdialysis, shortly after the local administration of GHB through the dialysis probe. A year later, Hechler *et al.* [5] confirmed the work of Maitre *et al. in vivo* and extended it to an enhancement of K^+-induced DA release *in vitro* following the incubation of rat striatal slices with GHB. Recently, Nissbrandt and his colleagues also reported a marked increase in the dialysate output of DA following the intrastriatal administration of GHB through the dialysis probe [6]. Finally, both a major review [7] and a recent research article [8] have indicated that GBH stimulates DA release. All of the above suggest that GBH may possibly *not* inhibit DA release as previously thought but rather stimulate it, at least *in vivo*.

The present study was undertaken to examine the discrepancy between studies showing an excitatory effect of GHB on central DA release and those reporting an inhibition. A secondary goal of the study was to investigate whether the effect impact of GHB on DA release might be affected by the route of administration used *in vivo*.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing 250–300 g were used throughout. All

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§ *Abbreviations:* DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GHB, γ-hydroxybutyrate; HPLC-EC, high performance liquid chromatography with electrochemical detection; HVA, homovanillic acid.

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animals were housed at constant room temperature (23°) and maintained on a 12-hr light–dark cycle. Food and water were available *ad libit*. The animals were randomly assigned to one of four groups as shown in Table 1 below.

ANESTHETIZED PROTOCOL. Animals (250–350 mg) were anesthetized with a low dose of urethane (1.5 g/kg, i.p.) and subsequently mounted in a sterotaxic frame (Kopf Instruments, Inc., Tujunga, CA). Following exposure of the skull, a small craniotomy was made over the neostriatum and a microdialysis probe was lowered into the striatum at stereotaxic coordinates AP:+1.0, L:3.0, relative to Bregma; and V:6.0 measured from the dural surface [9]. The dialysis probe had an outer diameter of 500 μm, a length of 3.0 mm, and a molecular weight cutoff of 20,000 (Carnegie Medicin CMA/12, Stockholm, Sweden). A period of 90 min was allowed for equilibration, prior to collecting baseline samples. Body temperature was monitored throughout using a rectal probe and telethermometer (Yellow Springs Instruments, Yellow Springs, OH).

UNANESTHETIZED PROTOCOL. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. Following exposure of the skull, a small craniotomy was performed at the coordinates given above. A guide cannula was secured to the skull using Repair Material Cement (Dentsply, New York, NY) with skull screws used to increase the hold. A period of 90 min was allowed for equilibration. Body temperature was monitored throughout surgery using a rectal probe and telethermometer (Yellow Springs Instruments) and was maintained at 37.0 ± 0.2°. After surgery, the animals were given 3–7 days to recover prior to their inclusion in the study.

On the day of the experiment, all animals were habituated to the dialysis chamber for at least 20 min prior to probe insertion. The dialysis probe described above was then inserted, and dialysis perfusion fluid (Ringer's solution) was infused at 5 μL/min for 90 min prior to the beginning of the experiment.

Three randomly selected animals were pretreated with urethane (1.5 g/kg, i.p.) and three with saline. As an injection control, they were then injected with saline, and subsequently monitored for the level of extracellular striatal DA in their dialysate for twelve 10-min intervals. The striatal DA release in all six animals was statistically identical (basal DA levels for saline: 2.26 ± 0.25 pmol; urethane: 2.58 ± 0.29 pmol). Moreover, striatal DA release in all of the 72 intervals monitored was statistically identical for all six animals. Therefore, data from the six animals were grouped together and used as a control group for purposes of comparison with groups 2, 3, and 4.

TABLE 1. Treatment groups

Group no.	Pretreatment dose	Treatment dose	Route of administration	N
1	Saline/Urethane (0.9%)/(1.5 g/kg)	Saline (0.9%)	Intraperitoneal	6
2	Saline (0.9%)	GHB (500 mg/kg)	Intraperitoneal	5
3	Urethane (1.5 g/kg)	GHB (500 mg/kg)	Intraperitoneal	5
4	Urethane (1.5 g/kg)	GHB (500 mg/kg)	Subcutaneous	5

Microdialysis

In both protocols, the dialysis probe was perfused with a degassed, filtered artificial cerebrospinal fluid (147 mM NaCl; 1.2 mM $CaCl_2$; 1.0 mM $MgCl_2$; 4.0 mM KCl, pH adjusted to 7.4) at a flow rate of 5 μL/min. Following insertion of the dialysis probe and a 90-min equilibration period, baseline samples were collected until a stable baseline was achieved, a process requiring a minimum of 30 min. The baseline was calculated by averaging the values from three samples collected immediately before drug administration. The mean of these values was set at 100%. The animals were then injected with either saline (controls) or 500 mg/kg of GHB (sodium salt; Sigma), and the experiment was continued for 120 min. Samples were collected at 10-min intervals in a microcentrifuge tube containing a 10-μL solution of 0.6 M perchloric acid, 0.01% sodium metabisulfite, and 1.0 μM 3,4-dihyroxybenzylamine (as an internal standard). Extracellular DA was then measured by microdialysis *in vivo*.

HPLC

The DA, DOPAC, and HVA contained in 50 μL of dialysate was quantified by reverse phase HPLC-EC. The compounds were separated on a reverse phase 100 mm × 3.2 mm (inner diameter) Biophase II column (Bioanalytical Systems, West Lafayette, IN). The mobile phase (consisting of filtered, degassed 0.10 M sodium phosphate, 0.10 mM EDTA, 0.10 mM octyl sodium sulfate, and 6% methanol, v/v pH adjusted to 3.50) was delivered at 1 mL/min. Oxidation of the compounds was performed at +0.7 Volts versus an Ag/AgCl reference electrode. The data are expressed as a percentage of the mean baseline value determined by dialysis samples collected prior to drug injection after correcting for recovery. Recoveries for individual probes ranged between 7 and 10% at room temperature.

Statistical Analysis

The data used for statistical analysis was in the form of percent baseline. Significance of the data was determined by a two-way ANOVA for repeated measures, including a comparison of subject means for each group across time. A one-way ANOVA was also done for each time period across all four groups. Finally, a one-way ANOVA was implemented for each time period to reveal significant differences for each group relative to the control group. Data were graphed as the mean and standard error of each group per time interval.

RESULTS

A two-way ANOVA for repeated measures revealed a highly significant ($P < .001$) difference between the group means over time relative to their effect on striatal DA release *in vivo* (Table 2).

A one-way ANOVA of all four groups for each time interval was also implemented. This also disclosed significant differences in striatal DA release *in vivo* between the four groups, relative to every time period in which release was monitored, with the exception of the first time period (Table 3). This latter was probably due to GHB brain levels being insufficient to produce a significant difference in striatal DA release relative to the dialysis DA output of control animals, after only 10 min of GHB administration. It is noteworthy that the significance of the differences between the groups tapered off over the last three time intervals (90–120 min), probably for a similar reason (i.e. as brain levels of GHB diminished for each group toward the end of the second hour, less heterogeneity would be expected in their effects on DA release).

GHB Administration in Awake Animals

Marked differences were observed in the effect of GHB (500 mg/kg) on the extracellular levels of DA in the neostriatum of conscious rats relative to anesthetized animals. In awake animals, GHB produced no effect on DA release until the second 10-min interval at which time a significant 18% decline in DA release was monitored ($P < .01$). Striatal DA release continued to fall significantly thereafter until a maximum decrease of just over 40% occurred 60 min after GHB administration. A slow recovery commenced immediately thereafter. However, DA release did not return to basal or saline control levels throughout the 2 hr of its monitoring, although the significance of the inhibition of release seen during the last three intervals decreased from $P < 0.01$ (intervals 2 through 9), to $P < 0.05$ (intervals 10 and 12; Table 4 and Fig. 1).

Rats were anesthetized with the minimum dose of urethane necessary to maintain a constant state of anesthesia (1.5 g/kg, i.p.). The anesthetic did not alter striatal DA release, as (1) the levels of DA release in conscious and anesthetized animals were not significantly different prior to the administration of GHB (as described in Materials and Methods), and (2) DA release in saline and urethane-pretreated animals subsequently administered saline (hereafter referred to as control animals) was the same across all intervals measured. Urethane obliterated the inhibitory effect of GHB on striatal DA release irrespective of whether GHB was administered subcutaneously or intraperitoneally (Figs. 2 and 3).

TABLE 2. Two-way ANOVA for repeated measures: Group means over time

Source of variance	SS	MS	DF	F	P
Within group	40621.47	2389.50	17		
Between group	64770.70	21590.23	3	9.04	< 0.001

GHB Administration in Anesthetized Animals

GHB ADMINISTERED INTRAPERITONEALLY AFTER URETHANE. Urethane pretreatment followed by intraperitoneal injections of GHB resulted in DA release that was close to that seen in control animals over all intervals monitored. During the first six intervals, the mean levels of DA release in GHB-treated animals were slightly below control levels, with a maximum decrease of 8.5% seen during the sixth interval. Mean DA levels gradually increased over the next six intervals until they exceeded control levels by a maximum of 12% by the last interval. However, the dialysis output of striatal DA levels did not fall or rise significantly below basal or control levels during any of the intervals measured after intraperitoneal GHB administration (Fig. 2).

GHB ADMINISTERED SUBCUTANEOUSLY AFTER URETHANE. Urethane pretreatment followed by subcutaneous injections of the same dose of GHB produced mean elevations of DA release across all twelve intervals. The increases ranged from 4 to 34% over control levels (Fig. 3). Significant increases in striatal DA release following the subcutaneous administration of GHB were monitored during the second, third and eleventh intervals (20–30, and 100–110 min after GHB administration; Table 5).

DISCUSSION

The present study was undertaken to determine whether GHB stimulates striatal DA release *in vivo* as suggested by several reports [3–8] or whether it inhibits this release as indicated by the results of numerous biochemical studies conducted *in vivo*, *ex vivo* and *in vitro* [10–30].

In addition to the biochemical studies cited above [10–30] there are numerous pharmacological and behavioral studies demonstrating an inhibitory effect of GHB and its substrate γ-butyrolactone release-dependent behavioral responses [31–37].

Further evidence that GHB inhibits central DA release is provided by the nature of the behavioral effects induced by GHB, which are strikingly similar to those induced by drugs inhibiting DA release. Such effects include akinesia, hypokinesia, sedation, catalepsy and loss of the righting reflex. All of these effects can be reversed by drugs stimulating DA release [2, 35–37]. Moreover, GHB inhibits the DA-release-dependent behaviors induced by indirect DA-releasing agonists irrespective of whether they are impulse independent (e.g. *d*-amphetamine [32–34] or impulse dependent (e.g. methylphenidate [35]).

Our results indicate that GHB (500 mg/kg) inhibits striatal DA release *in vivo* in awake but not in anesthetized animals. The present study is the first to our knowledge to

TABLE 3. One-way ANOVA of all four groups for each time interval, with (3,17) degrees of freedom

Interval	SS	Error SS	MS	Error MS	F	P
0–10	561.956	1891.864	187.319	111.286	1.68322	NS
10–20	1693.194	1560.704	564.398	091.806	6.14772	0.01
20–30	3940.695	1350.956	1313.565	079.468	6.52949	0.01
30–40	3562.767	2781.965	1187.589	163.645	7.25710	0.002
40–50	5567.361	4480.611	1855.787	263.565	7.04109	0.01
50–60	7889.305	7285.524	2629.768	428.560	6.13629	0.005
60–70	9858.471	7908.381	3286.157	465.199	7.06398	0.01
70–80	9947.185	5993.915	3315.728	352.583	9.40410	0.001
80–90	10527.218	9868.820	3509.072	580.519	6.04472	0.005
90–100	7154.464	8699.093	2384.822	511.711	4.66048	0.02
100–110	6519.929	6342.996	2173.310	373.117	5.82473	0.05
110–120	6569.117	8195.072	2189.723	482.063	4.54240	0.02

examine the effects of GHB on striatal DA release *in vivo* in the complete absence of any anesthetic. Some studies have examined the effect of GHB on striatal [^{3}H]-DA release [3, 30]. We avoided labeling the DA pool with tracer [^{3}H]-DA as a number of studies have consistently questioned the ability of the labeled amine to accurately reflect the effect of drugs on release of the endogenous neurotransmitter [38–41]. A GHB dose of 500 mg/kg was chosen as this was the dose to obtain the most dramatic increase in DA release of all the studies finding an increase in dialysate DA output after GHB (i.e. a > 600% increase within 40 min of GHB administration [4].

In the present study, the effect of GHB on DA release was examined in both awake and anesthetized animals as every study finding a GHB-induced enhancement of DA release did so in animals that were anesthetized, at least initially (3, 4–6]. Agents that interrupt neurotransmission pre- or post-synaptically produce an enhanced responsiveness to the transmitter when these agents are administered repeatedly (see Ref. 42). Indeed, this effect can occur within only a few hours following the acute interruption of striatal DA transmission by an indirect DA antagonist [42]. It therefore seemed possible that a reversal of the normally inhibitory effect of GHB on DA release might occur if one or more doses of an anesthetic other than GHB were to precede a high dose of GHB [43]. Some anesthetics significantly enhance striatal DA release while others do not, as discussed below. Consequently, the nature of any modification of the normally inhibitory effect of GHB on DA release would likely be determined by the specific anesthetic combined with GHB.

TABLE 4. One-way ANOVA for the effect of GHB (500 mg/kg, i.p.) on striatal DA release in awake animals relative to saline controls, with (1,9) degrees of freedom

	One-way ANOVA	
Interval (min)	F value	P value
0–10	2.65153	NS
10–20	11.36479	< 0.01
20–30	21.60801	< 0.002
30–40	19.71331	< 0.005
40–50	14.32599	< 0.01
50–60	10.65176	< 0.01
60–70	14.90211	< 0.01
70–80	17.55458	< 0.01
80–90	11.41705	< 0.01
90–100	5.18230	< 0.05
100–110	4.00990	NS
110–120	7.14022	< 0.05

Chéramy *et al.* [3] examined the effects of GHB [^{3}H]-DA release in the cat caudate and substantia nigra *in vivo*, using a push–pull cannula, and found a significant stimulation of DA release in both regions. All animals were under halothane anesthesia. However, halothane significantly increases and prolongs striatal DA release *in vivo*, in both the rat and cat [30, 44–50]. It is therefore possible that the increase in DA release observed by Chéramy and his coworkers was the result of an interaction between halothane and GHB.

Maitre *et al.* [4] and Hechler *et al.* [5] also observed increases in striatal DA release following GHB administration. While this increase may have been due to GHB *per se*, it is more likely that the concentration of Ca^{2+} in their dialysate was a primary factor in the increased DA release they observed. The dialysate solution has been demonstrated to be an important determinant of DA release in the striatal DA system. Numerous studies [51–54] have shown that Ca^{2+} concentrations higher than 2.3 mM result in significantly higher basal DA levels. Of equal importance, these studies have also found pharmacologically altered drug activity under conditions where Ca^{2+} concentrations exceeded 2.3 mM. In the studies of Maitre *et al.* [4] and Hechler and co-workers [5] the dialysate contained a concentration of Ca^{2+} that was 1.5-fold *greater* than 2.3 mM and *ca.* 3-fold greater than that used in the present study (3.4 vs 1.2 mM Ca^{2+}).

Nissbrandt *et al.* [6] recently observed a significant increase in DA output in the dialysate following the local intrastriatal administration of GHB through the dialysis

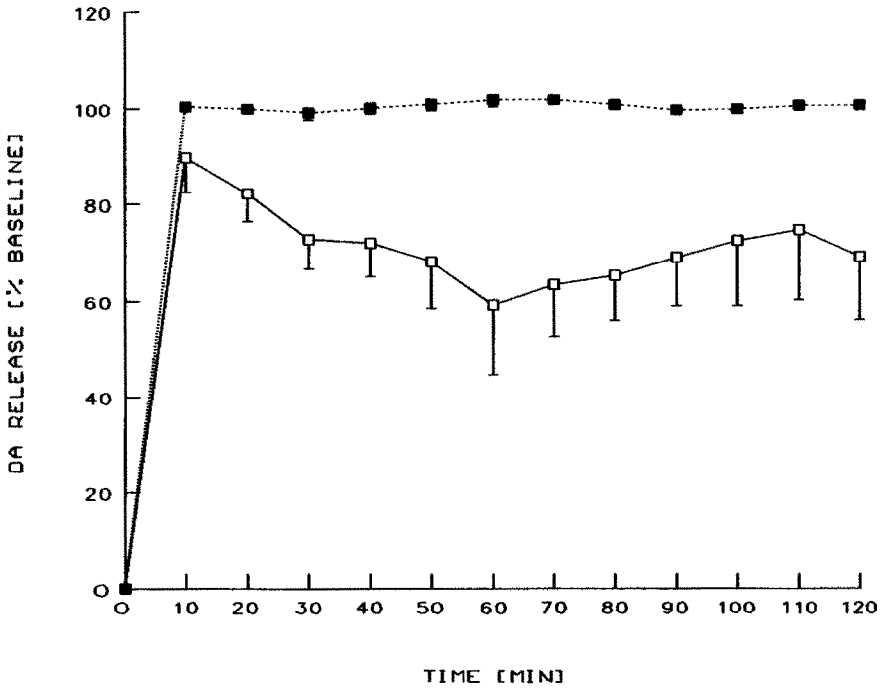

FIG. 1. Effect of GHB (500 mg/kg, i.p.) on striatal DA release *in vivo* in awake animals. Key: (■) saline/urethane + saline; (□) saline + GHB (500 mg/kg), N = 5. Values are means ± SEM. The basal level of DA for the control group was 2.54 ± 0.11 pmol for saline and 2.46 ± 0.09 pmol for urethane (1.5 g/kg, i.p.). The basal level of DA for GHB in awake animals was 2.5 ± 0.30 pmol.

probe. However, they maintained their rats under chloral hydrate anesthesia (400 mg/kg i.p. with additional doses added s.c. as needed). Chloral hydrate significantly alters striatal DA release *in vivo* [10, 55, 56] and its alteration of DOPAC and HVA efflux is also strikingly different from that induced by GHB [57]. It is therefore possible that the increase in DA release observed by Nissbrandt *et al.* [6] was due to the interaction of GHB with this anesthetic, which has been shown repeatedly to reverse the effects of both DA antagonists and agonists on central DA release [57–62]. Nissbrandt *et al.* [6, 7] have also proposed that GHB abolishes the burst activity in nigral DA neurons, and this has significance for the terminal efflux of DA. However, chloral hydrate anesthesia in the dose used by these authors has been found to markedly inhibit burst activity in nigral DA neurons by itself, quite independent of the presence of any other drug [63]. Thus again, the observations of Nissbrandt *et al.* [6] may have been seriously confounded by the presence of the anesthetic they used in combination with GHB.

A significant GHB-induced enhancement of striatal DA

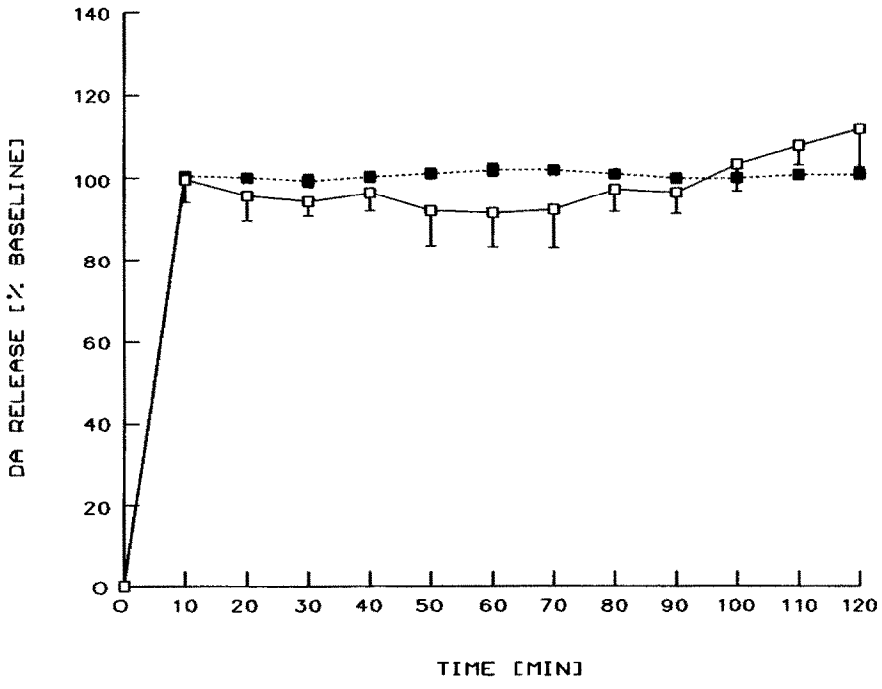

FIG. 2. Effect of GHB (500p mg/kg, i.p.) on striatal DA release *in vivo* in animals anesthetized with urethane (1.5 g/kg, i.p.). (■) saline/urethane + saline N = 6; and (□) urethane + GHB (500 mg/kg), N = 5. Values are the means + SEM, respectively. The basal level of DA for the control group appears in the legend of Fig. 1. The basal level of DA for GHB (500 mg/kg i.p.) in urethane-anesthetized animals was 2.08 ± 0.10 pmol.

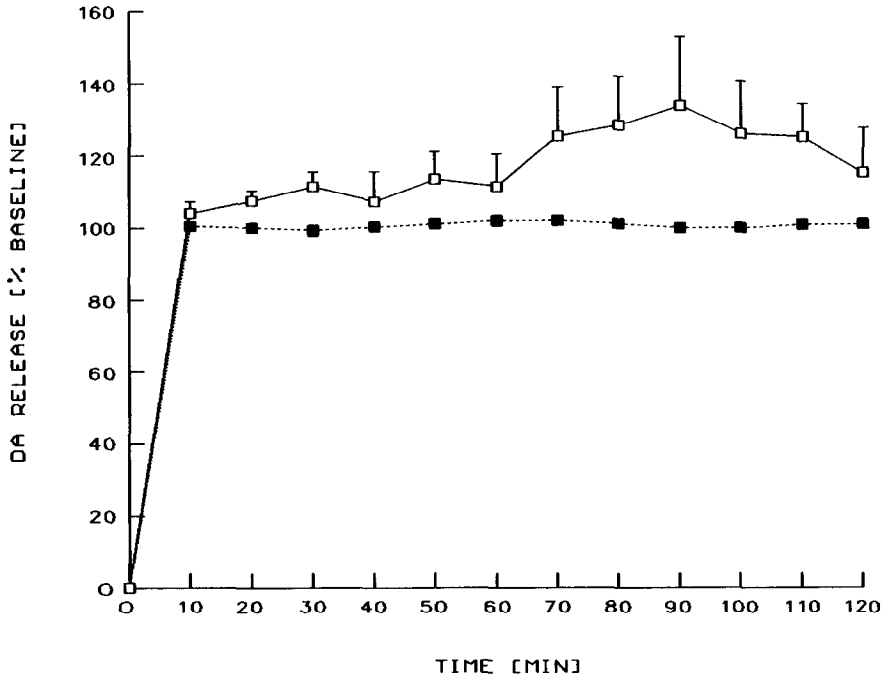

FIG. 3. Effect of GHB (500 mg/kg, s.c.) on striatal DA release *in vivo* in animals anesthetized with urethane (1.5 g/kg, i.p.) Key: (■) saline/urethane + saline N = 6; and (□) urethane + GHB (500 mg/kg, s.c.), N = 5. Values are means ± SEM. The basal level of DA for the control group appears in the legend of Fig. 1. The basal level of DA for GHB (500 mg/kg, s.c.) in urethane-anesthetized animals was 2.27 ± 0.20 pmol.

release was also observed in the present study in anesthetized animals, in agreement with the reports of Chéramy *et al.* [3], Maitre *et al.* [4], Hechler *et al.* [5] and Nissbrandt *et al.* [6]. Although the dose of urethane used did not alter DA release in the absence of GHB in common with a previous study [65] its interaction with GHB still resulted in a significant albeit modest increase in striatal DA release. It is therefore likely that the use of an anesthetic that *does* enhance DA release significantly would have resulted in a far more dramatic GHB-induced increase.

Urethane was found to exert a significantly greater reversal of the inhibitory effect of GHB on DA release when GHB was given subcutaneously versus intraperitoneally. This is very likely due to the greater increase in GHB blood levels seen after subcutaneous injection at the back of the neck (where an arterial plexis exists in the rat) relative to an intraperitoneal injection [65].

In conclusion, awake animals habituated to the dialysis procedure to minimize stress, and administered a high (500 mg/kg) dose of GHB, evidenced an inhibition of striatal DA release in consonance with previous biochemical and behavioral evidence. Recent work by Engberg and Nissbrandt [66] implicates $GABA_B$ receptors in this effect, although this finding is not unequivocal [67]. The present results also indicate that the inhibitory effect of high (≥500 mg/kg) doses of GHB on striatal DA release may be reversed completely by pretreatment with an anesthetic. However, it is also possible that discrepancies between the present study and the compelling studies cited above are not explicable in terms of the use of anesthetic or the concentration of Ca^{2+} in the dialysis fluid, but are due to other differences in methodology.

TABLE 5. One-way ANOVA for the effect of GHB (500 mg/kg, s.c.) on striatal DA release in anesthetized animals relative to saline controls, with (1,9) degrees of freedom

	One-way ANOVA	
Interval (min)	**F value**	***P* value**
10–20	7.48573	< 0.05
20–30	9.24927	< 0.05
100–110	8.06209	< 0.05

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