Br. J. Pharmac. (1973), **47**, 408–414.

Inhibition by γ-hydroxybutyrate of chlorpromazine-induced increase in homovanillic acid

R H. ROTH

Departments of Pharmacology and Psychiatry, Yale University School of Medicine and Connecticut Mental Health Center, New Haven, Connecticut 06510 U.S.A.

Summary

1. γ-Hydroxybutyrate and its lactone precursor, γ-butyrolactone, when administered in anaesthetic doses block the increase in the cerebral concentration of homovanillic acid normally observed after administration of chlorpromazine or haloperidol.

2. Other hypnotics such as glutethimide, urethane, chloralose, chloral hydrate and thiopentone do not have this ability even when administered in anaesthetic doses.

3. The ability of γ-hydroxybutyrate to block the neuroleptic-induced increase in cerebral homovanillic acid is not due to a reduction in body temperature since a similar effect is observed in rats treated under conditions (32° C) in which body temperature remains normal.

4. Possible mechanisms for this action of γ-hydroxybutyrate are discussed.

Introduction

γ-Hydroxybutyrate (GHB), the alkaline hydrolysis product of γ-butyrolactone (GBL) is a normal metabolite which occurs in mammalian brain in a concentration of 1–4 nmol/g (Roth & Giarman, 1970). When administered parenterally in large doses GHB or GBL produce a state of behavioural depression and a subsequent loss of the righting reflex in the rat. Recent experiments have indicated that administration of 'sleep' producing or anaesthetic doses of GHB or the lactone form, GBL, cause a large increase in the concentration of dopamine in the brain with little or no effect on brain noradrenaline, 5-hydroxytryptamine or γ-aminobutyric acid (Gessa, Vargiu, Crabai, Boero, Caboni & Camba, 1966 ; Roth & Surh, 1970 ; Giarman & Schmidt, 1963). This increase in the concentration of dopamine occurs primarily in brain areas normally rich in endogenous dopamine and appears morphologically to be restricted to the nerve terminals or varicosity portion of the dopamine containing neurones (Aghajanian & Roth, 1970). It has been suggested that this increase in dopamine is a result of a GHB-induced change in the utilization or release of dopamine from the neurones (Roth & Surh, 1970 ; Roth, 1972). This theory is further supported by the observation that rats treated with GBL plus probenecid at first have a lower brain concentration of homovanillic acid than rats treated only with probenecid, indicating that although more dopamine can be formed and/or retained by the brain, less is initially being metabolized (Roth & Surh, 1970 ; Roth, 1971). In addition, other studies have demonstrated that GBL can significantly decrease the amount of dihydroxyphenylacetic acid (DOPAC) found in rat striatum for periods up to 1 hour after GBL administration (Walters & Roth,

1972). However, 90 min after the administration of GHB, when the concentration of the drug and dopamine in the brain begin to decrease, the concentration of homovanillic acid and DOPAC rise rapidly. Preliminary studies in this laboratory have also indicated that administration of anaesthetic doses of GBL to rats antagonizes the increase in homovanillic acid and DOPAC normally produced in the subcortex by chlorpromazine (Roth, 1971 ; Roth, 1972). Subanaesthetic doses of GBL were found to be ineffective in blocking this increase in homovanillic acid (HVA).

The purpose of the work described here was twofold. First to determine if this effect of GHB in antagonizing the chlorpromazine-induced increase in HVA is specific for GHB and related active compounds or if it is a general property of all compounds possessing hypnotic or anaesthetic properties. Second, to see if GHB can also block the increase in HVA produced by another, structurally unrelated, neuroleptic drug, such as haloperidol.

Methods

Drug administration

All drugs were administered by the intraperitoneal route. When solubility permitted, drugs were administered in an aqueous solution. The following drugs were exceptions. Haloperidol (1 mg/kg) was dissolved in 0·1% solution of ascorbic acid and chlorpromazine (25 mg/ml) was dissolved in an aqueous solution containing 2 mg/ml of NaCl and 2% benzyl alcohol. The chlorpromazine solution was diluted 1:2·5 with distilled water before injection.

In one series of experiments, chlorpromazine (10 mg/kg) or haloperidol (5 mg/kg) was administered to male Sprague Dawley rats (Charles River Inc.) intraperitoneally and the concentration of HVA determined in the subcortex 90 minutes later. The increase in HVA produced by this treatment was compared to the increase observed when rats were administered a combination of a neuroleptic drug plus one of a group of various hypnotic drugs in an anaesthetic dosage. The effect of the hypnotic drug alone on the HVA concentration in the subcortex was also determined. The antagonism exerted by various hypnotic drugs on the neuroleptic-induced increase in HVA concentration was calculated by the following formula:

Per cent blockade of the neuroleptic-induced increase in HVA=

$$100-\left[\frac{(A-B)}{(C-D)}\times 100\right]$$

Where:

A=HVA concentration in rats treated with a hypnotic plus a neuroleptic

B=HVA concentration in rats treated with a given hypnotic

C=HVA concentration in rats treated with a neuroleptic alone

D=HVA concentration in untreated rats.

A second group of experiments was performed in which the rats were maintained in a 32° C environment after drug treatment in order to avoid any undue hypothermia as a result of the combined drug treatment.

Homovanillic acid determination

Rats were killed 1·5 h after administration of chlorpromazine (10 mg/ml) or haloperidol (5 mg/ml). The brains were rapidly excised and the subcortex, basal

ganglia, diencephalon and midbrain dissected out and immediately frozen on dry ice. The proteins were then precipitated by homogenization with four volumes of 0·4 M perchloric acid, and the extracts centrifuged to remove the precipitated protein. The precipitates were washed with an additional 2 ml of 0·4 M perchloric acid, recentrifuged and C^{14}-homovanillic acid (prepared enzymatically by incubation of dihydroxyphenylacetic acid with C^{14}-*S*-adenosylmethionine and catechol-*O*-methyl transferase (cf. Roth & Surh, 1970)) was added to the pooled supernatants as an internal standard. HVA was then extracted and analysed as described previously by Roth (1971) and Roth & Surh (1970). In all experiments 2 rat brain subcortical regions were pooled for each analysis. Statistical analyses were performed according to the procedure outlined by Snedecor (1956).

Rectal temperature measurements

The rectal temperatures of treated rats maintained at ambient temperature (22° C) or at 32° C were periodically monitored via probes connected to a Telethermometer model 46TUO obtained from Yellow Springs Instrument Company.

Results

Experiments in which tropolone, an inhibitor of catechol-*O*-methyl transferase (Belleau & Burba, 1961, 1963) was administered to rats demonstrated that inhibition of dopamine metabolism resulted in a rapid reduction in the concentration of subcortical HVA. In addition, pretreatment with tropolone completely blocked the

TABLE 1. *Effect of tropolone on the chlorpromazine-induced increase in homovanillic acid (HVA) in rat subcortex*

Treatment*	*n*	HVA (ng/g)
None	4	202±11
Chlorpromazine (10 mg/kg)	8	670±22
Tropolone (50 mg/kg)	3	<50
Tropolone+chlorpromazine	4	<50

* Rats were maintained in a 32° C environment for 90 min before killing. Rats were killed 90 min after chlorpromazine and 100 min after tropolone. All drugs were administered intraperitoneally. *n*=Number of individual experiments. Results are expressed as the mean ± the standard error of the mean.

TABLE 2. *Effect of γ-butyrolactone (GBL) on the chlorpromazine- and haloperidol-induced increase in homovanillic acid (HVA) in the rat subcortex*

Treatment*	*n*	HVA ng/g	HVA increase over respective control ng/g	% Blockade †
None	23	146± 5	—	—
Chlorpromazine (10 mg/kg)	13	581±34	435	—
Haloperidol (5 mg/kg)	8	607±67	461	—
GBL (750 mg/kg)	4	183±25	37	—
Chlorpromazine+GBL	4	274±21	91	79
Haloperidol+GBL	8	272±52	89	80

* Rats were killed 90 min after administration of drugs. HVA was measured in two pooled subcortices as described previously (Roth, 1971). *n*=Number of individual experiments. †% Blockade of the neuroleptic induced increase in HVA was calculated by the following formula:

$$100-\left[\frac{A-B)}{(C-D)}\times 100\right]$$

Where: A=HVA concentration in GBL+neuroleptic-treated rats; B=HVA concentration in GBL-treated rats; C=HVA concentration in neuroleptic-treated rats; D=HVA concentration in untreated rats.

increase in HVA normally observed after administration of chlorpromazine (Table 1).

When GBL, the precursor of GHB, was administered in anaesthetic doses simultaneously with chlorpromazine, the accumulation of HVA normally observed after chlorpromazine administration was also blocked. This ability of GBL was not limited only to the chlorpromazine-induced increase in HVA since the HVA increase produced by another structurally unrelated neuroleptic agent, haloperidol, was also antagonized by GBL (Table 2).

Other hypnotic drugs structurally unrelated to GHB such as methyprylone, glutethimide, urethane, chloralose and chloral hydrate, in anaesthetic doses, had little or no effect on the accumulation of HVA after chlorpromazine administration even at ambient temperature (22° C) (Table 3). However, it was observed that chloralose

TABLE 3. *Effect of several different hypnotics on the chlorpromazine-induced increase in homovanillic acid (HVA)*

Treatment	*n*	HVA (ng/g)	HVA increase over respective control (ng/g)	% Blockade*
None	23	146± 5	—	—
Chlorpromazine (10 mg/kg)	13	581±34	435	—
Methyprylone (300 mg/kg)	3	139±19	—	—
Methyprylone+chlorpromazine	4	492±78	353 NS	19
Glutethimide (200+100 mg/kg)	3	151±12	—	—
Glutethimide+chlorpromazine	4	466±18	315†	28
Urethane	3	141±15	—	—
Urethane+chlorpromazine	3	598±30	457 NS	0
Chloralose	3	320±24	—	—
Chloralose+chlorpromazine	4	687±39	367 NS	16
Chloral hydrate (400+200 mg/kg)	3	222±22	—	—
Chloral hydrate+chlorpromazine	4	622±12	400 NS	8
Pentobarbitone (60 mg/kg)	3	180±16	—	—
Pentobarbitone+chlorpromazine	4	394±17	214‡	51

Rats were killed 90 min after drug administration. *n*=Number of individual experiments. † Significantly different from chlorpromazine-treated rat, $P<0.05$. ‡ Significantly different from chlorpromazine-treated rat, $P<0.01$. NS=Not significantly different from chlorpromazine-treated rat, $P<0.05$. * % Blockade of the chlorpromazine-induced increase in HVA was calculated by the following formula:

$$100-\left[\frac{(A-B)}{(C-D)}\times 100\right]$$

Where: A=HVA concentration in GBL+chlorpromazine-treated rats; B=HVA concentration in GBL-treated rats; C=HVA concentration in chlorpromazine-treated rats; D=HVA concentration in untreated rats.

and chloral hydrate administered alone, in contrast to the other hypnotics studied, produced small but significant increases in HVA. In these experiments conducted at ambient temperature (22° C), it was observed that the combined administration of chlorpromazine, together with GBL or other hypnotic drugs often led to a 2–3 degree drop in body temperature within 90 minutes. In view of this observation some studies were repeated in which the rats were maintained in a 32° C environment from the time of administration of chlorpromazine or haloperidol until the experiment was terminated by killing the animal. When rats were maintained in a 32° C environment even the combined drug treatment (i.e. hypnotic plus neuroleptic drugs) did not result in a significant lowering of body temperature. Under these conditions GBL and GHB still blocked the chlorpromazine-induced increase in HVA by more than 80%, pentobarbitone by 43%, and thiopentone had no significant effect (Table 4). It was further observed in these experiments that untreated rats maintained at 32° C for 90 min had a significantly higher concentration of HVA in the

TABLE 4. *Effect of γ-butyrolactone (GBL), γ-hydroxybutyrate (GHB), pentobarbitone and thiopentone on the chlorpromazine-induced increase in homovanillic acid (HVA)*

Treatment	*n*	HVA (ng/g)	HVA increase over respective control (ng/g)	% Blockade*
None	9	194±12	—	—
GHB (1,100 mg/kg)	4	376±41	182	—
GBL (750 mg/kg)	4	260±28	66	—
Pentobarbitone (60 mg/kg)	4	214±52	20	—
Thiopentone (40+20 mg/kg)	3	244±10	54	—
Chlorpromazine (10 mg/kg)	12	666±34	472	—
Chlorpromazine+GHB	4	475± 7	99	79
Chlorpromazine+GBL	7	310±18	50	89
Chlorpromazine+pentobarbitone	4	483±19	269	43
Chlorpromazine+thiopentone	4	704±16	460	2

Rats were maintained in a 32° C environment for 90 min before killing. Rats were killed 90 min after injection of chlorpromazine. *n*=Number of individual experiments. * % Blockade of the chlorpromazine-induced increase in HVA was calculated as in Table 3.

TABLE 5. *Effect of pretreatment with γ-butyrolactone (GBL) on the chlorpromazine and haloperidol-induced increase in homovanillic acid*

Treatment	*n*	HVA (ng/g)	HVA increase over respective control (ng/g)	% Blockade*
None	9	194±12	—	—
Chlorpromazine (10 mg/kg)	6	604±46	410	—
Haloperidol (5 mg/kg)	10	570±20	376	—
GBL (13 meq./kg)	5	211±10	19	—
Chlorpromazine+GBL	7	190±19	—	100
Haloperidol+GBL	3	269±37	58	85

Rats were maintained in a 32° C environment for 90 min before killing. Rats were killed 90 min after injection of chlorpromazine or haloperidol. GBL was administered i.p. in a divided dose of 8·7 mEquiv/kg 105 min before killing and 4·3 mEquiv/kg 60 min before killing. Results are expressed as mean±S.E.M. *n*=Number of individual experiments. * % Blockade of the neuroleptic induced increase in HVA was calculated as in Table 2.

subcortex than rats kept at ambient temperature. If rats were pretreated with GBL 15 min prior to the administration of chlorpromazine or haloperidol an even more complete block in the neuroleptic-induced increase in HVA in the subcortex was observed (Table 5).

Discussion

It has been known for several years that chlorpromazine and other neuroleptics produce a large increase in brain concentrations of dopamine metabolites such as homovanillic acid and dihydroxyphenylacetic acid while they have little effect on the endogenous concentration of the parent catecholamine, dopamine (Andén, Roos & Werdinius, 1964 ; Carlsson & Lindqvist, 1963 ; Laverty & Sharman, 1965). The ability of chlorpromazine and haloperidol to increase dopamine metabolites has been attributed to the ability of these neuroleptics to increase the synthesis and turnover of dopamine in the central nervous system (Nybäck & Sedvall, 1968). Very recently, Bunney, Walters, Roth & Aghajanian (1972) have demonstrated that chlorpromazine and haloperidol cause an increase in the rate of firing of the dopamine containing neurones located in the zona compacta of the substantia nigra. It is quite likely that it is this increase in activity of dopamine neurones caused by neuroleptic drugs which is ultimately the cause of the increase in the neuronal release and subsequent metabolism of dopamine observed after treatment of animals with these agents.

Since experiments in this laboratory had suggested that GHB was mediating its effects on dopamine neurones at least in part by blocking the release of dopamine, it was of interest to determine if GHB could also antagonize the increase in the concentration of HVA normally produced by chlorpromazine and haloperidol.

Initial experiments with tropolone indicated that when HVA formation was blocked by inhibition of catechol-*O*-methyl transferase that the chlorpromazine-induced increase in HVA was completely blocked. These observations suggested that the increase in HVA produced by chlorpromazine was a result of new synthesis of HVA. Treatment of rats with GHB or the lactone precursor, GBL, likewise almost completely antagonized the ability of chlorpromazine and haloperidol to increase the concentration of HVA. This action was independent of any alteration in body temperatures. Since GHB has been shown to have no inhibitory effect on the dopamine metabolizing enzymes (Gessa, Crabai, Vargiu & Spano, 1968), this block in the chlorpromazine-induced increase in HVA was presumed to be the result of the ability of GHB to block dopamine release from the neurone, thus preventing its subsequent metabolism to HVA. This ability of GHB and its analogues to block the neuroleptic-induced increase in HVA seems to show some specificity in that most other hypnotics tested lack this ability. Pentobarbitone was the exception, since at high doses it produced a smaller but significant antagonism of the chlorpromazine-induced increase in HVA concentration.

The mechanism by which GHB blocks the release of dopamine from dopamine containing neurones is not completely elucidated. *In vitro* studies conducted in this laboratory (Bustos & Roth, 1972a and b) have indicated that GHB added to the medium superfusing striatal slices results in about a 30% inhibition of the release of newly synthesized dopamine from the slices during depolarization with potassium ions. These results suggest that GHB might be exerting some direct action on the release of dopamine from the nerve terminals in the striatum. However, the contribution of this direct effect on the release of dopamine from the nerve terminals to the effects of GHB observed *in vivo* is at present difficult to ascertain. Recent experiments in this laboratory, more relevant to the *in vivo* situation, have demonstrated that GBL administered in doses of 250–750 mg/kg inhibits the firing of dopamine neurones in the substantia nigra (Walters, Aghajanian & Roth, 1972). The end result of an inhibition of neuronal activity in the nigro-neostriatal pathway would be the suppression of transmitter release at the nerve terminals. In view of recent observations on the effect of chlorpromazine and other neuroleptic drugs (Bunney *et al.*, 1972) and GHB (Walters *et al.*, 1972) on unit firing of dopamine neurones, the most likely explanation for the observed biochemical effects described above is that GHB prevents the neuronal feedback activation of dopamine neurones normally produced by the neuroleptic drugs and in this way antagonizes the chlorpromazine-induced release of dopamine and the resultant accumulation of HVA. Unit recording experiments are in progress to test this possibility.

This work was supported in part by a grant from the United States Public Health Services—MH-14092.

The author would like to thank Miss Janice Abele for her excellent technical assistance.

The author would also like to thank the following pharmaceutical companies for their generous supply of some of the drugs used in these experiments: Ciba Pharmaceutical Company for the glutethimide, Hoffman-La Roche Inc. for the methyprylone and McNeil Laboratories for the haloperidol.

REFERENCES

AGHAJANIAN, G. K. & ROTH, R. H. (1970). Gamma-hydroxybutyrate-induced increase in brain dopamine: localization by fluorescence microscopy. *J. Pharmac. Exp. Ther.*, **175**, 131–138.

ANDÉN, N.-E., ROOS, B. E. & WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci.*, **3**, 149–158.

BELLEAU, B. & BURBA, J. (1961). Tropolones: A unique class of potent non-competitive inhibitors of S-adenosylmethionine-catechol methyl transferase. *Biochem. Biophys. Acta*, **54**, 195–196.

BELLEAU, B. & BURBA, J. (1963). Occupancy of adrenergic receptors and inhibition of catechol-*O*-methyl transferase by tropolones. *J. med. Chem.*, **6**, 755–759.

BUNNEY, B. S., WALTERS, J. R., ROTH, R. H. & AGHAJANIAN, G. K. (1972). Dopaminergic neurons: Effect of antipsychotic drugs and amphetamine on single cell activity. *J. Pharmac. exp. Ther.* (in press).

BUSTOS, G. & ROTH, R. H. (1972a). Effect of gamma-hydroxybutyrate on the release of monoamines from the rat striatum. *Br. J. Pharmac.*, **44**, 817–820.

BUSTOS, G. & ROTH, R. H. (1972b). Release of monoamines from the striatum and hypothalamus. Effect of gamma-hydroxybutyrate. *Br. J. Pharmac.*, **46**, 101–115.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine and haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmac. Toxicol.*, **20**, 140–144.

GESSA, G. L., CRABAI, F., VARGIU, L. & SPANO, P. F. (1968). Selective increase of brain dopamine induced by gamma-hydroxybutyrate: study of the mechanism of action. *J. Neurochem.*, **15**, 377–381.

GESSA, G. L., VARGIU, L., CRABAI, F., BOERO, G. C., CABONI, F. & CAMBA, R. (1966). Selective increase of brain dopamine induced by gamma-hydroxybutyrate. *Life Sci.*, **5**, 1921–1930.

GIARMAN, N. J. & SCHMIDT, N. J. (1963). Some neurochemical aspects of the depressant action of gamma-butyrolactone on the central nervous system. *Br. J. Pharmac. Chemother.*, **20**, 563–568.

LAVERTY, R. & SHARMAN, D. F. (1965). Modifications by drugs of the metabolism of 3,4-dihydroxyphenylanaline, noradrenaline and 5-hydroxytryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759–772.

NYBÄCK, H. & SEDVALL, G. (1968). Effect of chlorpromazine on accumulation and disappearance of catecholamines formed from tyrosine-C^{14} in brain. *J. Pharmac. exp. Ther.*, **162**, 294–301.

ROTH, R. H. (1971). Effect of anesthetic doses of gamma-hydroxybutyrate on subcortical concentrations of homovanillic acid. *Eur. J. Pharmac.*, **15**, 52–59.

ROTH, R. H. (1972). Striatal dopamine and gamma-hydroxybutyrate. In: *International Encyclopaedia of Pharmacology and Therapeutics*, Vol. 25, 'The Pharmacology of the Extrapyramidal System' (in press).

ROTH, R. H. & GIARMAN, N. J. (1970). Natural occurrence of gamma-hydroxybutyrate in mammalian brain. *Biochem. Pharmac.*, **19**, 1087–1094.

ROTH, R. H. & SURH, Y. (1970). The mechanism of the gamma-hydroxybutyrate-induced increase in brain dopamine and its relationship to 'sleep'. *Biochem. Pharmac.*, **19**, 3001–3012.

SNEDECOR, G. W. (1956). *Statistical Methods*, 5th ed. Iowa State College Press, Ames.

WALTERS, J. R. & ROTH, R. H. (1972). Effect of gamma-hydroxybutyrate on dopamine and dopamine metabolites in the rat striatum. *Biochem. Pharmac.*, **21**, 2111-2121.

WALTERS, J. R., AGHAJANIAN, G. K. & ROTH, R. H. (1972). *Dopaminergic Neurons: Inhibition of Firing by Gamma-hydroxybutyrate*, Proc. Fifth Int. Cong. Pharmacol., p. 246, No. 1472.

(*Received July* 14, 1972)