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EFFECT OF AMINOOXYACETIC ACID, THIOSEMICARBAZIDE AND HALOPERIDOL ON THE METABOLISM AND HALF-LIVES OF GLUTAMATE AND GABA IN RAT BRAIN

GODFREY G. S. COLLINS

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, England

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Abstract—The activity of the enzymes glutamate decarboxylase (GAD) and GABA-2-oxoglutarate transaminase (GABA-T), the endogenous GABA and glutamate levels and the half-lives ($t_{\frac{1}{2}}$ values) of radioactive GABA and glutamate have been measured in the brains of untreated rats and in those injected with aminooxyacetic acid (AOAA), thiosemicarbazide (TSC) and haloperidol. The effect of the drugs *in vitro* on a purified preparation of glutamate dehydrogenase (GDH) was also measured.

AOAA profoundly inhibited GABA-T, did not inhibit GAD and in *in vitro* experiments activated GDH. Brain glutamate levels were unaffected by the drug as was the half-life for glutamate. Brain GABA levels were elevated after AOAA and the rate of disappearance of radioactive GABA slowed.

TSC did not significantly alter either GABA or glutamate brain levels but did significantly inhibit GAD and GDH activities. In addition, TSC had no effect on the half-life of either GABA or glutamate.

Haloperidol was an effective inhibitor of GDH *in vitro* and reduced brain glutamate concentrations *in vivo*. Moreover, the rate of disappearance of radioactive GABA was increased whereas that of glutamate was decreased. The drug had no effect on GAD and GABA-T activities or on brain levels of GABA.

It is suggested that the behavioural effects of haloperidol might in part be due to actions on the metabolism of GABA and glutamate. Interaction of TSC with glutamate metabolism might be a factor in seizure-provoking activity of the drug.

EVIDENCE that GABA and glutamate are neurotransmitter substances within the CNS continues to accumulate[1,2] and is supported by the effects of drugs known to interfere with the metabolism of these transmitter candidates. The major pathway for GABA metabolism, for example, involves transamination with 2-oxoglutarate in a reaction catalysed by the enzyme 4-aminobutyrate: 2-oxoglutarate aminotransferase (EC 2.6.1.19; GABA-T); inhibition of this step *in vivo* by aminooxyacetic acid (AOAA) elevates brain GABA levels[3–5] with a concomitant sedation of the animals. The convulsant hydrazides, on the other hand, may preferentially inhibit glutamate decarboxylase (L-glutamate-1-carboxylase; EC 4.1.1.15: GAD) thereby reducing brain concentrations of GABA.[6–9] Little attention has been paid to the effects of such drugs on the dynamic aspects of GABA metabolism although it has recently been reported that the convulsant bicuculline, a pharmacological antagonist of GABA[10,11] is a weak *in vitro* inhibitor of GABA-T and also prolongs the half-life ($t_{\frac{1}{2}}$ value) of GABA in rat brain.[12] There is also a paucity of reports of the effects of compounds on either steady state or dynamic aspects of glutamate metabolism.

In this study, the actions of three drugs on glutamate and GABA brain levels and their turnover times and also on the activities of GAD, GABA-T and glutamate dehydrogenase (GDH; L-glutamate: NAD(P) oxidoreductase (deaminating); EC 1.4.1.3) have been investigated. The drugs used were AOAA, thiosemicarbazide (TSC) and haloperidol (γ-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-*p*-fluorobutyrophenone), the first two as they have fairly well defined biochemical sites of action, the third as it it is not only an effective inhibitor of GABA uptake[13] but is a potent inhibitor of GDH[14] and all three as they cause marked changes in behavioural patterns.

MATERIALS AND METHODS

Animals. White male Wistar rats weighing 140–175 g were used throughout the experiments and allowed free access to food and water.

Administration of drugs. TSC (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) was dissolved in 0·9% w/v saline to give a concentration of 1 mg/ml; AOAA (Aldrich Chemical Company Inc., Milwaukee, Missouri, U.S.A.) was in buffered saline (0·9% w/v, pH 6·2) at a concentration of 20 mg/ml; haloperidol was made up in unbuffered saline containing 1% v/v lactic acid at a final concentration of 1 mg/ml. All drugs were administered by intraperitoneal injection.

Determination of free GABA and glutamate levels. The concentration of GABA in brain was estimated by the enzymic fluorimetric method described by Kravitz and Potter.[15] Groups of 6 rats were injected with the appropriate drug and killed by cervical fracture either immediately (control groups) or at various times thereafter. The extraction of the amino acids and the subsequent estimation of GABA has been described previously.[16] Briefly, a portion of an acid extract was incubated with a preparation of GABase (for preparation of this enzyme, see Scott and Jakoby[17]) and the reduced NADP measured fluorimetrically.[15] Endogenous brain glutamate levels were also estimated by an enzymatic procedure.[17] In principle, the glutamate in the acid extracts was oxidized to 2-oxoglutarate using a purified preparation of beef liver GDH (Boerhinger Mannheim GmbH) and the reduced acceptor molecule, acetyl $NADH_2$, estimated fluorimetrically. The experimental procedure was identical with that described by Young and Lowry.[18] Both GABA and glutamate levels have been expressed as μmole/g wet wt of tissue.

Measurement of enzyme activities. Both brain GAD and GABA-T were measured after treatment of rats with AOAA, TSC and haloperidol. Groups of 6 rats were injected with the appropriate drug and killed either immediately (control groups) or at various later times. Brains were immediately weighed and homogenized in 0·3 M sucrose containing 0·01 M 2-mercaptoethanol, 0·5% v/v of the nonionic detergent Triton X-100 and 10 mg/l EDTA.[16] Brain GAD activity was estimated by following the rate of $^{14}CO_2$ release from 1-^{14}C-DL-glutamate (Radiochemical Centre, Amersham, U.K.). The experimental procedure was based on that devised by Roberts and Simonsen[19] as previously described[16] but modified by the omission of pyridoxal-5′-phosphate from the incubation mixture. The GABA-T activity of the homogenates was measured using the radiochemical method described by Hall and Kravitz[20] using 2,3-^{3}H-GABA (New England Nuclear Corp., Dreieichenhain, West Germany) as substrate except that the B_6 coenzyme was omitted from the incubation mixtures. The activities of both these enzymes have been expressed as mmol substrate metabolized/kg wet wt of tissue/hr.

The effect of the *in vitro* addition of AOAA, TSC and haloperidol on GDH activity has also been estimated. Two assay procedures have been used, one employing glutamate and the second 2-oxoglutarate as substrate. In the first, the incubation mixture consisted (final concentrations) of glutamate (50 mM), NAD (10 mM) and glutamate dehydrogenase (1 μg/ml; Boehringer Mannheim GmbH) in 2 ml of Tris–HCl buffer (0·1 M, pH 8·4). The rate of reduction of the NAD was followed at 37° at 0·5 min time intervals in a spectrophotometer. In the second procedure, the incubation mixture consisted of (again final concentrations) 2-oxoglutarate (10 mM), $NADH_2$ (5 mM), NH_4Cl (50 mM) and GDH (1 μg/ml) in 2 ml of the Tris–HCl buffer. In this case, the rate of oxidation of the reduced coenzyme was measured in a spectrophotometer. The initial rates of change in extinction at 340 nm were estimated graphically and results have been expressed as percentages of control activities. Drugs (in 0·1 ml) were added to the mixtures either 20 min prior to or at the same time as the substrate. Suitable control samples were prepared for each drug at the two preincubation times and control samples were always run simultaneously with the test incubates. Because of practical difficulties, the GDH activity of brain extracts has not been estimated.

Estimation of the half-lives of glutamate and GABA in rat brains. Glutamate and GABA $t_{\frac{1}{2}}$ values were estimated by measuring the rates of disappearance of the radioactive compounds after intracisternal administration. U-^{14}C-L-glutamic acid (270 mCi/mmole; The Radiochemical Centre, Amersham, England) was supplied in aqueous solution containing 2% v/v ethanol which was then made isotonic by the addition of solid sodium chloride. Maximum volumes of 40 μl (containing 2·5 μCi of ^{14}C-glutamic acid) were injected into the cisterna magna of rats lightly anaesthetized with ether. Where appropriate, drugs were administered simultaneously and the rats killed by cervical fracture various times later (0·5, 1, 2, 3, 4, 6 and 8 hr). Similarly, other groups of rats were injected with 10 μl of an isotonic solution containing 5 μCi of 2,3-^{3}H GABA and killed 0·5, 1, 2, 4, 6 and 8 hr later. The brains were rapidly weighed and homogenized in 0·2 N HCl to give a final concentration of 100 mg wet wt of tissue/ml. After storage overnight the proteins were precipitated by heating in boiling water bath and amino acids separated by ion exchange chromatography using Amberlite CG-120, Type II, 200 mesh, H^+ form[21] (for details of procedures, see Collins[16]). The eluates from the columns were evaporated to dryness, redissolved in 5 ml of distilled water and passed through 4 × 0·5 cm columns of Dowex-AG-1X2 resin, 100–200 mesh, acetate form; glutamine and GABA passed through whereas the acidic amino acids glutamate and aspartate remained bound. The columns were washed with 5 ml of water, the washings added to the eluate, mixed with 10 ml of "Instagel" (Packard Instrument Company) and the radioactivity counted in a scintillation spectrometer. Where appropriate, the ^{14}C-labelled acidic amino acids (glutamate and aspartate) were eluted from the Dowex resin with 7·5 ml of 0·2 N HCl; this eluate was also mixed with 10 ml of "Instagel" and the radioactivity measured. When known amounts of radioactive GABA and glutamate were added to brain homogenates and passed through the entire extraction procedure, recoveries of 77·8% ± 3·6[16] and 67·7% ± 5·3 respectively (mean of 10 experiments ± S.E.M.) were obtained. All results have been corrected for recovery and expressed as dis./min/g wet wt of tissue.

The purity of the final extracts was determined by paper chromatography using a butanol:acetic acid:water system (4:1:1 by vol.);[16] more than 90 per cent of the radioactivity in the "GABA" fraction was due to ^{3}H-GABA.[16] When paper chromato-

Table 1. Effect of aminooxyacetic acid (AOAA), thiosemicarbazide (TSC) and haloperidol on rat brain GABA and glutamate contents

Drug	Dose (mg/kg)	Time after injection (hr) 0	0·5	1	2	4	6	8
		GABA content (μmole/g)						
AOAA*	25	3·27 ± 0·31	3·71 ± 0·16	5·69 ± 0·35†	7·04 ± 0·42†	9·18 ± 1·48†	7·70 ± 0·60†	6·25 ± 0·23†
TSC	7·5	3·18 ± 0·41	3·34 ± 0·24	3·75 ± 0·43	2·80 ± 0·19	2·91 ± 0·26	2·57 ± 0·29	2·48 ± 0·31
Haloperidol	1	3·09 ± 0·28	2·28 ± 0·09‡	2·45 ± 0·34	2·13 ± 0·08‡	2·81 ± 0·31	2·19 ± 0·23‡	2·50 ± 0·17
		Glutamate content (μmole/g)						
AOAA	25	14·0 ± 1·70	14·7 ± 1·28	13·1 ± 1·48	13·4 ± 1·42	12·5 ± 1·20	15·7 ± 1·42	
TSC	7·5	13·5 ± 1·12	14·2 ± 1·60	13·6 ± 0·77	12·7 ± 0·56	13·4 ± 1·11	12·5 ± 0·48	
Haloperidol	1	13·6 ± 0·80	12·5 ± 0·35	10·9 ± 0·36‡	11·8 ± 0·35	11·7 ± 0·47	13·0 ± 0·53	

Groups of six rats were injected intraperitoneally with the appropriate drug and killed at various times. Results are averages ± S.E.M.

* Taken from Collins.[16] The brain levels of GABA and glutamate in untreated animals were 3·14 ± 0·17 and 13·7 ± 0·69 μmole/g respectively (mean of 10 experiments ± S.E.M.).

† Significantly different ($P < 0·005$) from zero time value.

‡ Significantly different ($P < 0·05$) from zero time value.

graphy of the acid eluate was carried out, less than 8 per cent of the radioactivity was due to compounds other than ^{14}C-glutamate (mean of 10 experiments). The results have not been corrected for this small amount of contamination.

The dis./min/g of brain tissue for each amino acid was plotted on a $\log_{10}$ scale against time after injection in hours. The regression coefficients of the lines were calculated and the half lives estimated using the formula $t_{\frac{1}{2}} = (\log_{10}2)/\text{slope}$. Significant differences between the slopes were measured by applying the Student's t-test to the regression coefficients. An estimate of the turnovers in μmole/g/hr was made using the calculated $t_{\frac{1}{2}}$ values and the endogenous tissue levels of the amino acids. When drug administration resulted in significant alteration in the tissue contents of either GABA or glutamate an estimate of turnover was made using the $t_{\frac{1}{2}}$ value and the mean amino acid concentration over the appropriate time period.

RESULTS

Brain GABA and glutamate levels after administration of AOAA, TSC and haloperidol. After injection of AOAA (25 mg/kg) the expected rise in brain GABA content occurred which reached a maximum 3–4 hr after drug administration (Table 1). Injection of TSC (7·5 mg/kg) caused a small increase in GABA content 1 hr after administration but the level then fell and at 8 hr was less than that at zero time. Haloperidol (1 mg/kg) caused a small but significant fall in brain GABA content ($P < 0{\cdot}05$). Of the three drugs investigated, only haloperidol affected brain glutamate levels, there being a significant fall ($P < 0{\cdot}05$) 1 hr after administration (Table 1).

The effect of AOAA, TSC and haloperidol on the activities of GAD, GABA-T and GDH. Groups of rats were injected with one or other of the drugs and GAD and GABA-T activities in homogenates of the brains estimated (Table 2). The activity of both enzymes was less than that measured in the presence of pyridoxal-5′-phosphate.[16] After AOAA, there was no significant change in GAD activity but the GABA-T activity was profoundly inhibited for at least 8 hr. On the other hand, TSC appeared to inhibit both enzymes although only GAD inhibition was statistically significant ($P < 0{\cdot}05$). Haloperidol was without effect on either GAD or GABA-T activity (Table 2). The effect of all three drugs on a purified preparation of GDH is shown in Table 3. As expected,[14] haloperidol proved to be a potent inhibitor, a concentration of 0·2 mM causing approximately 50 per cent inhibition of activity. However, the equal potency of TSC in inhibiting GDH activity was unexpected whereas semicarbazide, even at high concentrations, was ineffective. High concentrations of AOAA were found to activate GDH, especially if the drug and enzyme were preincubated prior to addition of the substrate. The effects of TSC and AOAA and of haloperidol and AOAA were found to be mutually antagonistic when incubated together in the reaction mixture.

The effects of AOAA, TSC and haloperidol on GABA and glutamate half-lives in whole rat brain. The disappearance of tracer doses of radioactive GABA from rat brain consists of a fast and slow component;[16] none of the drugs investigated significantly altered the turnover times of the rapid efflux phase (Table 4). However, AOAA significantly prolonged and haloperidol significantly accelerated the slower second phase ($P < 0{\cdot}05$). Both AOAA and haloperidol altered brain GABA levels (see Table 1) and in order to achieve approximations for the GABA turnovers, the mean GABA

TABLE 2. EFFECT OF THE ADMINISTRATION *in vivo* OF AMINOOXYACETIC ACID (AOAA), THIOSEMICARBAZIDE (TSC) AND HALOPERIDOL ON THE ACTIVITIES OF GAD AND GABA-T

Drug	Dose (mg/kg)	Enzyme	Enzyme activities (mmole/kg/hr) at various times after drug administration				
			0 hr	1 hr	2 hr	4 hr	8 hr
AOAA	25	GAD	31·8 ± 2·6	26·3 ± 2·9	38·6 ± 3·9		37·5 ± 5·5
		GABA-T	82·6 ± 5·1	9·3 ± 1·7*	6·1 ± 0·9*		5·2 ± 1·3*
TSC	7·5	GAD	33·1 ± 2·6	20·7 ± 2·6†	25·2 ± 1·8†	24·3 ± 4·9	26·7 ± 3·3
		GABA-T	86·4 ± 3·4	77·8 ± 7·2	79·4 ± 1·9	73·2 ± 7·9	81·3 ± 6·2
Haloperidol	1	GAD	34·3 ± 4·6		36·3 ± 4·7	37·2 ± 3·4	35·9 ± 4·6
		GABA-T	84·7 ± 2·9		86·7 ± 5·7	89·2 ± 4·9	83·9 ± 8·2

Groups of five rats were injected, killed at various times, homogenates of the brains made and enzyme activities measured in the absence of pyridoxal-5′-phosphate (see Materials and Methods). Each value is the mean activity ± S.E.M. The corresponding GAD and GABA-T activities for untreated animals were 31·7 ± 2·9 and 88·7 ± 7·7 mmole/kg/hr respectively (mean of eight experiments ± S.E.M.).

* Significantly different ($P < 0.001$) from zero time activity.

† Significantly different ($P < 0.05$) from zero time activity.

TABLE 3. EFFECTS OF VARIOUS DRUGS ON THE ACTIVITY OF A PURIFIED PREPARATION OF GLUTAMATE DEHYDROGENASE (GDH)

Drug	Final concentration (mM)	Preincubation time (min)	Control activity and range (%)	No. of experiments
Haloperidol	0·1	0	62 (51–73)	4
	0·2	0	46 (40–60)	4
	0·5	0	30 (18–51)	3
	2	0	19 (11–31)	4
	2	20	17 (12–22)	2
Aminooxyacetic acid	0·9	0	103 (91–112)	4
	0·9	20	95 (82–101)	4
	9·0	0	136 (123–151)	4
	9·0	20	185 (152–210)	4
Thiosemicarbazide	0·11	0	71 (63–81)	3
	0·22	0	41 (30–52)	3
	0·33	0	33 (26–35)	4
	1·1	0	13 (8–18)	2
	11·0	0	14 (9–21)	4
	11·0	20	10 (7–13)	2
Semicarbazide	8·7	0	102 (98–104)	2
	8·7	20	100 (94–106)	2

GDH activity was measured using either glutamate or 2-oxoglutarate as substrate. Suitable control incubations were carried out for each drug concentration and preincubation time. Results are expressed as mean per cent of control activity together with the range of values.

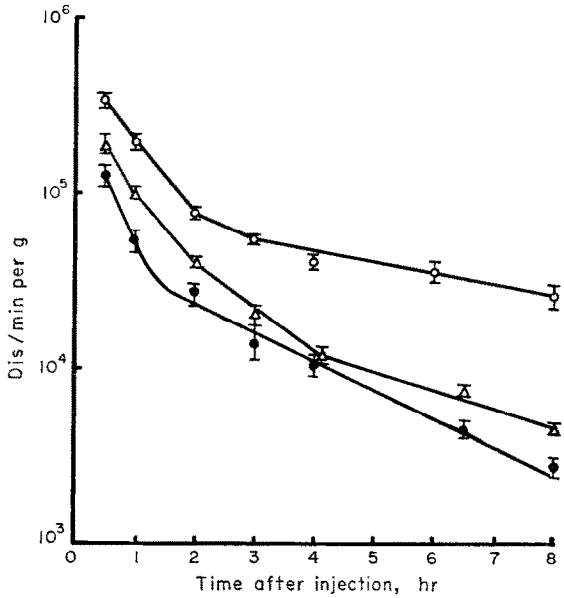

FIG. 1. The disappearance of total radioactivity (○), total radioactive amino acids (△) and radioactive glutamate (●) after the intracisternal injection of 2·5 μCi of U-^{14}C-L-glutamate. Each point is the mean of ten experiments ± S.E.M. The half-life of ^{14}C-glutamate was calculated as 2·62 hr.

TABLE 4. EFFECT OF AMINOOXYACETIC ACID (AOAA), THIOSEMICARBAZIDE (TSC) AND HALOPERIDOL ON THE HALF-LIVES AND TURNOVER RATES OF GABA AND GLUTAMATE IN RAT BRAIN

			GABA					
			Time after injection of ^{3}H-GABA					
			Between 0·5 and 2 hr			Between 4 and 8 hr		
Drug	Dose (mg/kg)	No. of animals	$t_{\frac{1}{2}}$ (hr)	95% confidence limits	Turnover (μmole/g/hr)	$t_{\frac{1}{2}}$ (hr)	95% confidence limits	Turnover (μmole/g/hr)
None*		8	0·45	0·29–0·66	3·49	3·10	2·92–3·36	0·506
AOAA	25	6	0·71	0·52–0·98	(3·81)	10†	7·2–13·1	(0·386)
TSC	7·5	6	0·53	0·34–0·66	2·96	3·52	2·91–4·12	0·446
Haloperidol	1	6	0·32	0·23–0·47	(3·59)	1·68†	1·45–1·81	(0·822)

			Glutamate		
			Between 1 and 8 hr after injection of ^{14}C-glutamate		
			$t_{\frac{1}{2}}$ (hr)	95% confidence limits	Turnover (μmole/g/hr)
None		10	2·62	2·31–3·12	2·62
AOAA	25	6	2·21	1·84–2·61	3·10
TSC	7·5	6	2·38	1·88–2·69	2·88
Haloperidol	1	6	5·94†	4·72–6·21	(1·01)

Drugs were administered immediately prior to the intracisternal injection of the rats with the appropriate radioactive amino acid. The $t_{\frac{1}{2}}$ values were measured by following the rates of disappearance of either the ^{3}H-GABA or ^{14}C-glutamate (see Materials and Methods). Turnover rates were calculated from half-life and estimated size of the corresponding amino acid pool (see Table 1). Those values in parentheses are only approximations having been calculated from the estimated mean GABA content over the appropriate time period (see text).

* Results taken from Collins.[16]

† Significantly different ($P < 0·05$) when compared with untreated controls.

concentrations over the two efflux components were calculated and used to estimate the turnovers shown in parentheses (Table 4). Thus, although the $t_{\frac{1}{2}}$ after AOAA administration was 10 hr, because of the high tissue GABA content, turnover was little affected. Conversely, although haloperidol reduced brain GABA levels (see Table 1) turnover is approximately 60 per cent higher than that of untreated animals.

The disappearance of ^{14}C-glutamate from whole rat brain after intracisternal administration was essentially monophasic (Fig. 1). From the slope of the line, the $t_{\frac{1}{2}}$ value was calculated to be 2·6 hr and the turnover to be approximately 2·62 μmole/g/hr. The initial rapid flux of glutamate between 0·5 and 1 hr may represent efflux of radioactivity from extracellular spaces and for this reason has not been considered. Haloperidol retarded the efflux of glutamate, the turnover time increasing from 2·62 to 5·94 hr. Calculation of the approximate turnover rate suggests that after haloperidol, turnover was markedly reduced (Table 4).

DISCUSSION

Our understanding of the functions of glutamate and GABA in the CNS is far from complete. Until recently, it was assumed that glutamate[22] and GABA[13] were simply important intermediates in general metabolism. More recently, the suggestion that these amino acids might be neurotransmitters in the CNS[1,2] has led to a reassessment of the significance of the neurochemical and neurophysiological data available. It has been claimed[2] that the high O_2 requirement of the GABA–glutamate system might not be an indication of its importance in general metabolism but of the relatively large energy requirements necessary to maintain adequate amounts of these important transmitters. However, at present it is generally accepted that both GABA and glutamate occupy an important position in intermediary metabolism in addition to their postulated roles as neurotransmitters.

The compartmentation of metabolic events has received much attention in recent years. Both GABA and glutamate exist in multiple pools within the CNS,[24] each of which possesses its own particular influx and efflux rate constants.[25,26] Two types of compartmentation are recognized,[24] the first being described in anatomical and the second in biochemical terms. Both these classifications have been applied to the GABA-glutamate system; the larger pool is thought to be concerned with energy production[24,27,28] whereas the smaller component, which is probably confined to the nerve terminals,[29,30] is involved in a synthetic capacity and perhaps neurotransmission.[25,29] Although the constituents of both pools are in equilibrium, it is clearly important when interpreting the effects of drugs to know not only how but at which site those drugs are exerting their actions. In this study, the actions of AOAA, TSC and haloperidol have been measured on a series of parameters affecting the GABA–glutamate system.

Although AOAA is a potent inhibitor of GABA-T activity, other transaminases are also susceptible to inhibition by this drug.[5] Recent evidence[31] shows that the major site of action of AOAA is in the "energy forming" cycle rather than the smaller "transmitter" pool whereas glutamate[31] and GABA uptake[13] is unaffected. In the present results, the inhibition of GABA-T was accompanied by the expected rise in GABA content. In addition, the activity of GAD remained relatively unchanged whereas GDH activity at least *in vitro* was activated. The rise in GABA levels was

accompanied by a slowing of the rate of disappearance of ^{3}H-GABA suggesting that GABA-T activity has some role in regulating GABA turnover (Collins[16]). On the other hand, the $t_{\frac{1}{2}}$ value for glutamate was unaffected by the drug. The action of AOAA on GDH may be significant for this enzyme is preferentially located in the neuronal rather than the glial fraction;[32] any alteration in glutamate levels or turnover caused by modification of GDH activity may therefore represent "transmitter" rather than "metabolic" glutamate. However, AOAA had no effect on glutamate half-life in these experiments (see Table 4).

There has also been much speculation as to the site of action of TSC. It was originally thought that the drug preferentially inhibited GAD rather than GABA-T thereby reducing GABA levels and precipitating seizures.[4,6,33] Certain discrepancies[34] in the relationship between GABA content, GAD inhibition and onset of seizures prompted Sze and Lovell[35] to re-examine the situation and they concluded that *in vivo*, TSC was ineffective as an inhibitor of GAD. In the present experiments, however, even though sub-convulsive doses were used, GAD activity was significantly inhibited (see also Wood and Abrahams[9]) although GABA content was not significantly affected (see Tables 1 and 2). TSC has also been shown in the present series of experiments to be a potent inhibitor of GDH although neither glutamate levels nor turnover were affected. In addition, although GAD was also inhibited, the rate of disappearance of ^{3}H-GABA remained unaltered, providing support for the suggestion[9] that changes in GABA turnover are unlikely to be involved in the aetiology of TSC-induced seizures. It is clear from the present results that the seizure-provoking activity of this drug may involve disturbances not only in GABA metabolism but also in that of glutamate.

Haloperidol has at least two modes of action for not only is it a potent inhibitor of GDH[14] but in addition is one of the more effective inhibitors of GABA uptake.[13] This latter effect would tend to cause an increased turnover of GABA and in these experiments haloperidol significantly reduced the half-life of GABA. Inhibition of GDH *in vivo* might be expected to increase the availability of glutamate for conversion to GABA; in these experiments, however, haloperidol reduced both GABA and glutamate levels. On the other hand, TSC, an equally potent inhibitor of GDH *in vitro*, had no such effect on amino acid levels. Clearly, the results cannot be interpreted using such a simple approach.

Drugs affecting the GABA–glutamate system will have at least two possible major modes of action. Firstly, there are those drugs that might interfere with the formation, storage, release and metabolism of the fraction of the GABA–glutamate system concerned in transmission. Secondly, drugs could act on that fraction concerned with general metabolism. Of course, most drugs probably interact to a greater or lesser extent with both compartments. In addition, drugs acting preferentially on the smaller transmitter pools might cause profound changes within those pools that with the experimental techniques employed in the present work would be masked by the larger "metabolic" pools. Thus, although it is thought that AOAA acts mainly on the "metabolic" component[31] it is not known whether either TSC or haloperidol show any specificity of site of action. Until the significance of the different turnover rates of GABA and glutamate can be appreciated, it will be difficult to interpret the present results. However, the results suggest that the actions of haloperidol and AOAA on the half-lives of GABA and glutamate may be relevant in any consideration of the behavioural effects of these drugs. Similarly, inhibition of GDH by TSC *in vivo* may

help to explain some of the anomalous results described for the biochemical mechanisms involved in hydrazide-induced seizures. Future work must aim at producing drugs having specific biochemical sites of action so that it will be possible to relate behavioural with neurochemical actions.

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