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BIOCHEMICAL AND PHARMACOLOGICAL ACTIONS OF IMIDAZOLEACETIC ACID

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Abstract—The effects of imidazoleacetic acid (IMA) on glutamate metabolism and biogenic amine levels in mouse brain are contrasted with those of gamma-hydroxybutyric acid (GHB) and pentobarbitone. The data show (1) IMA, 400 mg/kgi.p., specifically increases GABA pool size without a concomitant increase in biosynthesis of GABA from glutamate. (2) Conversely, GHB, 500 mg/kgi.p., decreases the turnover rate of glutamate to GABA, without affecting the amino acid pool sizes. Pentobarbitone, 60 mg/kgi.p., does not have any effects on those aspects of glutamate metabolism studied. (3) IMA inhibits GABA-T, $K_i = 3{\cdot}4 \times 10^{-4}$ M. The inhibition is of a noncompetitive nature. (4) The level of the glutamine pool and its metabolism remain unaffected by all three drugs. (5) GHB increases dopamine 250 per cent and serotonin 125 per cent in brain stem. IMA also increased serotonin level by 70 per cent. Pentobarbitone did not have any consistent effects on biogenic amine levels in brain stem.

PARENTERAL administration of imidazoleacetic acid (IMA), a product of oxidative deamination of histamine[1] induces an hypnotic state in mice from which they can be aroused by tactile stimulation.[2] The hypnotic effect of IMA is overtly similar to that induced by administration of gamma-hydroxybutyric acid (GHB).[3] Although both drugs produce a somnolent state, IMA and GHB administrations are characterized by a progressive CNS excitation.[4] It has been reported that GHB occurs naturally in brain[5] and that it can be formed from GABA *in vivo*.[6] IMA, when applied iontophoretically onto cortical neurones closely resembles GABA in its hyperpolarizing effects both in potency and time course of action.[7,8] McGeer, McGeer and McLennan[9] earlier reported that IMA has an inhibitory action on the stretch receptor of crayfish.

Bachelard and Lindsay[10] presented evidence that neurotropic drugs and pentobarbitone reduced the incorporation of carbon from uniformly [^{14}C]labeled glucose into brain glutamate, glutamine and GABA. Godin, Mark and Mandel[11] have reported that glucose metabolism is markedly affected during somnolence induced by GHB; in particular, the turnover from glutamate to GABA and to glutamine is diminished, whereas the pool sizes of these amino acids do not differ from control values. This lack of effect on amino acid pool sizes after GHB has been substantiated

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by Margolis.[12] Further, it has been claimed that GHB specifically increases dopamine levels in brain.[13]

The present report compares the effects of IMA with GHB, when they are administered at doses sufficient to induce a somnolent state, on certain aspects of glutamate metabolism *in vivo*, and on biogenic amine levels in mouse brain. These effects are also contrasted with those following pentobarbitone anaesthesia.

MATERIALS AND METHODS

Animals. Male mice of an albino strain and weighing between 20–25 g were obtained from Chemical Defence Establishment, Porton Down, Salisbury, Wilts., England and used in all the experiments. Mice were killed by total immersion into liquid nitrogen. For investigations of glutamate metabolism, the mice were injected i.p. with 20 μCi [U-^{14}C]glucose 20 min prior to killing. It had initially been established that this time course provided optimal incorporation of the labelled carbon into the relevant brain amino acids.

Glutamate metabolism. The frozen whole brains were rapidly removed and placed immediately into 6 ml homogenizing tubes containing 2 ml of 80% ethanol (v/v). These were then placed in a boiling water bath for 1–2 min following which they were homogenized (TRI-R Pestle Tissue Homogenizer at 2000 rev/min for 1 min). The homogenates were kept at 4° for 12 hr to allow for extraction of the soluble intermediates. The supernatants were evaporated to dryness *in vacuo* and the residues made up to 0·5 ml with 80% ethanol. These solutions were slowly evaporated onto individual Whatman No. 4 chromatography grade paper and the soluble intermediates separated by two-dimensional chromatography. The radioactive intermediates were visualized by autoradiography and glutamate, glutamine and GABA were identified. The radioactivity in, and pool size of, the three amino acids were individually determined.[14] The ratio of the specific activities of GABA and glutamine to glutamate were taken as measures of GABA and glutamine production respectively.[15]

GABA amino-transferase (GABA-T) assay. A soluble enzyme extract was prepared from mouse brain by the method of Waksman and Roberts.[16] The assay conditions were based on the method of Hall and Kravitz,[17] but using [5-^{14}C]-2-ketoglutarate in place of [^{14}C]GABA. The incubations were performed in the following mixture: mercaptoethanol 2·0 μl; pyridoxal phosphate, 100 μg; GABA, 0·15–3·0 μmole; [5-^{14}C]-2-ketoglutarate (spec. act. 0·25 uCi/μmole), 2 μmole; borate buffer (pH 8·4); 200 μmole; enzyme extract, equivalent to 10 mg acetone powder. IMA, when present, was 3·0 mM. Incubations were carried out at 37° for 1 hr and the reaction stopped with 100 μl. 0·4 N perchloric acid. The precipitate was spun down and the supernatant applied to a column of Dowex 50 × 2 resin (200 mesh) in a column 0·5 cm diameter and 2 cm long. The glutamate was eluted with 0·1 N HCl and 1 ml of the elutant withdrawn for scintillation counting in 6 ml NE250 scintillation medium. Blank incubations were carried out in the absence of GABA in order to correct for the non-specific breakdown of 2-ketoglutarate. The specific activity of the enzyme preparation was found to be 0·48 μmole glutamate formed/hr/milligram protein.

Biogenic amine assays. The estimation of brainstem levels of serotonin, dopamine and noradrenaline utilized a modification of the technique described by Brownlee and Spriggs.[18] In the estimation of noradrenaline, half quantities of the reagents used

by these workers were employed. During the estimation of dopamine, the addition of 0·5 ml of 0·5 M phosphate buffer, pH 6·5, to 1 ml of the extract obviated the need for exact pH recording by a microelectrode. Ultraviolet irradiation of the sample prior to spectrophotofluorimetry was not performed.

Drug doses. The doses used and the times of administration prior to killing were as follows:

Pentobarbitone (nembutal)	60 mg/kg 15 min
GHB	500 mg/kg 30 min
IMA	400 mg/kg 20 min

All drugs were given i.p.; control mice received an equal volume of 0·9% physiological saline.

RESULTS

Drug effects on glutamate metabolism. The incorporation of ^{14}C into the total free glutamate pool and those of its metabolites in brain under the various conditions was measured 20 min after intraperitoneal injection of [U-^{14}C]glucose. The size of the total glutamate pool remained stable under all the conditions with the exception of IMA at 400 mg/kg, when a just significant decrease was measured ($P < 0·05$, Table 1). Under all other conditions the total glutamate pool size was within the range 8·46 ± 0·12* μM/g brain tissue. The size of the total glutamine pool was not significantly affected by any of the drugs at the dose levels used and remained within the range 6·63 ± 0·24 μM/g brain tissue. The total GABA pool increased significantly following IMA ($P < 0·001$, Table 1). Under all other conditions the total GABA pool size remained within the range 0·700 ± 0·009 μM/g brain tissue.

TABLE 1. FREE AMINO ACID LEVELS IN THE WHOLE BRAIN OF DRUG-TREATED MICE

	Controls (20)	Pentobarbitone 60 mg/kg (6)	GHB 500 mg/kg (6)	IMA 400 mg/kg (6)
Glutamate	8·24 ± 0·29	8·50 ± 0·92	8·27 ± 0·93	6·91 ± 0·32*
Glutamine	6·25 ± 0·37	6·11 ± 0·38	7·29 ± 0·11	7·29 ± 0·57
GABA	0·723 ± 0·041	0·680 ± 0·034	0·696 ± 0·041	1·60 ± 0·12†

Date reported as mean ± S.E.M. and expressed in μmoles/g wet wt. The number of determinations are shown in parentheses. Note the large increase in the GABA pool following administration of IMA compared with the relative stability of the amino acid pools under all other conditions.

* $P < 0·05$; † $P < 0·001$.

Compared to total pool sizes large variations from control values were detected in the specific activities of the three amino acids under all drug conditions (Table 2). In general, specific activities were significantly lower following drug administration than were control values. GHB and pentobarbitone administration was followed by significant, but uniform, decreases in all the specific activities measured, indicating a general decrease in glucose incorporation into the three amino acids. Following

* Mean ± standard error of the mean.

400 mg/kg IMA only the specific activity of GABA was decreased compared to control ($P < 0.001$, Table 2), the values for the other two amino acids remaining within 10 per cent of control.

TABLE 2. SPECIFIC ACTIVITIES OF FREE AMINO ACIDS IN WHOLE BRAIN FOLLOWING INTRAPERITONEAL INJECTION OF [U-^{14}C]GLUCOSE IN DRUG-TREATED MICE

	Controls (20)	Pentobarbitone (6) 60 mg/kg	GHB (6) 500 mg/kg	IMA (6) 400 mg/kg
Glutamate	4294 ± 283	2565 ± 312†	1815 ± 148‡	4035 ± 512
Glutamine	1474 ± 125	941 ± 141*	516 ± 56‡	1687 ± 286
GABA	1412 ± 125	876 ± 76*	315 ± 30‡	292 ± 45‡

Data reported as mean ±S.E.M. and expressed as cpm/μmole amino acid. Number of determinations shown in parentheses. Note the decrease in specific activity of GABA following the administration of IMA without a similar decrease in the other two amino acids. This specificity contrasts with the indiscriminate decreases in glucose incorporation following the administration of GHB or pentobarbitone.

* $P < 0.050$; † $P < 0.01$; ‡ $P < 0.001$.

Table 3 contains the GABA–glutamate ratios for total pool size and specific activity. The pool size ratios after administration of GHB or pentobarbitone showed little variation from control values, remaining within the range 0·0869 ± 0·0001.* At 400 mg/kg IMA the GABA–glutamate pool size ratio increased by 160 per cent ($P < 0.001$, Table 3).

TABLE 3. THE POOL SIZE AND SPECIFIC ACTIVITY RATIOS FOR GABA RELATIVE TO GLUTAMATE IN THE WHOLE BRAIN OF DRUG-TREATED MICE

	Control (20)	Pentobarbitone 60 mg/kg (6)	GHB 500 mg/kg (6)	IMA 400 mg/kg (6)
Pool size ratio	0·0883 ± 0·0042	0·0846 ± 0·0089	0·0877 ± 0·0079	0·2343 ± 0·0199†
Specific activity ratio	0·350 ± 0·033	0·374 ± 0·053	0·179 ± 0·022*	0·071 ± 0·006†

Ratio of pool size = GABA μmole/g vs. glutamate μmole/g.

Ratio of specific activity = cpm/μmole GABA vs. cpm/μmole glutamate.

Values expressed as means ±S.E.M. Number of determinations in parentheses. Note the marked decrease in GABA production following administration of IMA.

* $P < 0.05$; † $P < 0.001$.

The ratio of specific activity of GABA to glutamate during pentobarbitone anaesthesia did not vary significantly from the control value, but did decrease significantly following GHB ($P < 0.05$, Table 3). After the administration of IMA this specific activity ratio decreased fivefold compared to control ($P < 0.001$, Table 3).

The pool size and specific activity ratios for glutamine relative to glutamate remained relatively constant (Table 4), compared to the corresponding data for GABA. Under all the experimental conditions these pool size ratios were within the range 0·832 ± 0·022 and the specific activity ratios within the range 0·400 ± 0·021.

* As a ratio score no units are applicable.

TABLE 4. THE POOL SIZE AND SPECIFIC ACTIVITY RATIOS FOR GLUTAMINE RELATIVE TO GLUTAMATE IN THE WHOLE BRAIN OF DRUG-TREATED MICE

	Control (20)	Pentobarbitone 60 mg/kg (6)	GHB 500 mg/kg (6)	IMA 400 mg/kg (6)
Pool size ratio	0·790 ± 0·052	0·759 ± 0·071	0·900 ± 0·046	0·880 ± 0·032
Specific activity ratio	0·389 ± 0·024	0·454 ± 0·046	0·302 ± 0·019	0·428 ± 0·037

Ratio of pool size = glutamine μmole/g vs. glutamate μmole/g.
Ratio of specific activity = cpm/μmole glutamine vs. cpm μmole glutamate.
Values expressed as means ± S.E.M. Number of observations in parentheses. Note the consistent lack of effect of all three substances on glutamine metabolism.

To summarize these data, IMA substantially increased the total free GABA pool size, without a concomitant increase in the biosynthesis of GABA from glutamate. GHB, on the other hand, decreased the turnover rate of glucose carbon from glutamate to GABA, in terms of the specific activity ratios, without affecting the amino acid pool sizes. Pentobarbitone, apart from decreasing the incorporation of glucose carbon into all three amino acids, did not have any specific effects on those aspects of glutamate metabolism studied. The level of the total free glutamine pool and its metabolism remained relatively unaffected by all three drugs.

Inhibition of GABA-T by IMA. The inhibition of GABA-T by IMA was determined by calculating the effect of 3·0 mM IMA on the K_m (GABA) and the maximum velocity of the reaction. A Lineweaver–Burk plot was made and is shown as Fig. 1. It is seen from this figure that IMA did not alter the K_m (GABA) at the concentration used, but

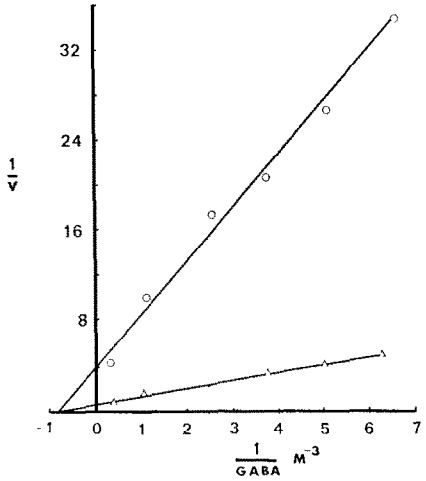

FIG. 1. Double reciprocal plot of the transamination of GABA by GABA-T. (△) incubations performed in the presence of 3 mM 2-ketoglutarate, (○) incubations performed in the presence of 3 mM 2-ketoglutarate and 3 mM IMA. Initial velocity is expressed as dis./min × 10^{-3} appearing/min in glutamic acid. The intersection of the two lines on the x axis indicated non-competitive inhibition by IMA. K_i (IMA) = 3·4 × 10^{-4} M; K_m (GABA) = 1·3 × 10^{-3} M.

significantly reduced the V_{max}. The inhibition is of a non-competitive nature and the K_i for IMA, calculated from the plot, is $3{\cdot}4 \times 10^{-4}$ M. GHB did not alter the activity of the enzyme at concentrations up to 5 mM.

Drug effects on biogenic amine levels. The effects of IMA, GHB and pentobarbitone on the levels of dopamine, noradrenaline and serotonin were assayed in the brainstem. Consistent effects were found with GHB (Table 5). At the dose level used GHB increased dopamine concentration from a control value of $3{\cdot}14 \pm 0{\cdot}42$ nmole/g wet wt to $10{\cdot}54 \pm 1{\cdot}18$ nmole/g wet wt ($P < 0{\cdot}001$), Table 5. Values are corrected for percentage recovery. GHB also increased the level of serotonin from $2{\cdot}11 \pm 0{\cdot}21$ nmole/g wet wt to $4{\cdot}65 \pm 0{\cdot}06$ nmole/g wet wt ($P < 0{\cdot}001$), see Table 5. As can be seen from the rest of the data in Table 5, pentobarbitone and IMA were either ineffective or produced inconsistent effects. Pentobarbitone did not produce any changes in the levels of biogenic amines measured in the brain stem. Following IMA serotonin increased by 70 per cent ($P < 0{\cdot}05$, Table 5). However, this increase in serotonin following IMA was much more variable than the greater increase measured after GHB administration. The level of noradrenaline under all the conditions in the investigation did not vary significantly from control levels.

TABLE 5. DRUG EFFECTS ON BIOGENIC AMINE LEVELS IN MOUSE BRAIN STEM

	Control (5)	Pentobarbitone 60 mg/kg (3)	GHB 500 mg/kg (3)	IMA 400 mg/kg (3)
Serotonin	$2{\cdot}11 \pm 0{\cdot}21$	$2{\cdot}09 \pm 0{\cdot}23$	$4{\cdot}65 \pm 0{\cdot}06$†	$3{\cdot}58 \pm 0{\cdot}42$*
Dopamine	$3{\cdot}14 \pm 0{\cdot}42$	$4{\cdot}21 \pm 0{\cdot}81$	$10{\cdot}54 \pm 1{\cdot}18$†	$3{\cdot}82 \pm 0{\cdot}12$
Noradrenaline	$2{\cdot}70 \pm 0{\cdot}32$	$3{\cdot}04 \pm 0{\cdot}23$	$1{\cdot}90 \pm 0{\cdot}23$	$3{\cdot}50 \pm 0{\cdot}78$

Data is given in nmole/g wet wt. Values are means ±S.E.M. Number of determinations are in parentheses. Note the marked increase in serotonin and dopamine following administration of GHB.

* $P < 0{\cdot}05$; † $P < 0{\cdot}001$.

DISCUSSION

Both IMA[19] and GHB[6] can be formed in brain and the possibility that either or both of these substances have metabolic functions in brain activity under normal conditions should not be overlooked. At the dose levels used in these experiments, GHB induced a somnolent state from which the animals could be aroused. IMA also produced somnolence from which arousal was possible, pentobarbitone acted as a general anaesthetic.

The drug effects on glutamate metabolism were measured in terms of total, free amino acid concentrations in whole brain and therefore interpretation of the results is limited since any change, or lack of change, reported may reflect adjustments in normal blood–brain levels of the compounds concerned or inter-adjustments in the heterogenous compartmentalization of glutamate metabolism and its products. In this context, [U-^{14}C]glucose was used as a substrate since it does lead to the preferential labeling of that metabolic pool of glutamate concerned with GABA synthesis.[20]

An overall decreased incorporation of brain glucose carbon into the free amino acid pools was measured following GHB and pentobarbital administration (Table 2),

however, there is evidence that GHB has far more specific effects on overall glucose metabolism than does pentobarbitone.[3] The present results directly substantiate the findings of Fleming and Lacourt[21] who compared the differential effects of GHB and pentobarbitone on brain energy metabolism. A number of other workers have found similar effects to those reported here following GHB administration, including the lack of effect on GABA pool size.[11,12,22] Godin *et al.*[11] further demonstrated a decrease in the specific activity of GABA relative to glutamate which the present findings substantiate (Table 3); although the corresponding decrease in glutamine specific activity, which they also reported, was not confirmed (Table 4).

IMA administration did not lead to a decreased incorporation of glucose carbon into glutamate and glutamine (Table 2), contrasting with the effects of the other two substances used. IMA also differed considerably from both GHB and pentobarbitone in its effects on glutamate/GABA metabolism. It was followed by a large increase in the GABA pool size (Table 1) and a concomitant decrease in GABA specific activity relative to that of glutamate (Table 3). These two effects were interpreted in terms of an inhibition by IMA of the enzyme GABA-T. This interpretation was substantiated (Fig. 1), IMA having a K_i of $3{\cdot}4 \times 10^{-4}$ M. This inhibition contrasts with the action of GHB, following which the GABA–glutamate ratio of specific activities, but not pool sizes, was also decreased, implying an inhibition of GAD. This latter conclusion has also been reached by other workers.[11] Further, the activity of GABA-T was not effected *in vitro* by GHB.

In view of the possible involvement of serotonin in the neurohumoral control of sleep,[23] it is interesting that both GHB and IMA lead to significant increases in the level of this biogenic amine in brain stem, while pentobarbitone anaesthesia produced no significant changes in the level of this or the other two amines measured (Table 5). Brodie, Shore and Pletscher[24] did not detect any change in brain serotonin level following pentobarbitone administration, but Anderson and Bonnycastle[25] have reported that many centrally acting drugs, including pentobarbitone, significantly elevate whole brain serotonin levels. The present results show that GHB increases the level of dopamine and that of serotonin significantly in the brain stem. The effect of GHB on dopamine levels in the present investigation is of the same magnitude as that reported by Gessa *et al.*[13]

The data reported in this investigation show gross differences in the action of the three hypnotics pentobarbitone, GHB and IMA on the intermediary metabolism of glutamate in whole brain and on the levels of biogenic amines in the brain stem of mice. Although it is evident from work in other laboratories[4] as well as our own that there is a marked similarity in the overt effects, behavioural and electroencephalographic, of IMA and GHB when administered to mice, these similarities were not reflected in those aspects of brain metabolism studied. However, the inhibition of GABA-T by IMA may indicate some inter-relation with GHB metabolism and experiments are now proceeding in which we hope to investigate this possible relationship further.

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