Br. J. Pharmac. (1982), **75**, 219–227

THE EFFECTS OF ANAESTHETICS ON THE UPTAKE AND RELEASE OF AMINO ACID NEUROTRANSMITTERS IN THALAMIC SLICES

T.J.G. KENDALL & M.C.W. MINCHIN[1]

Department of Physiology, University of Sheffield, Sheffield S10 2TN

1 The effect of thiopentone, methohexitone, urethane and ketamine on the uptake and release of γ-aminobutyric acid (GABA) and D-aspartate by rat thalamic slices has been investigated.

2 A high, supra-anaesthetic concentration of methohexitone increased the uptake of both D-aspartate and GABA.

3 None of the anaesthetics used had any detectable effect upon the spontaneous release of either amino acid.

4 Urethane and ketamine had no effect upon the K^+-stimulated release of either amino acid.

5 Methohexitone and thiopentone produced a biphasic dose-response on the K^+-stimulated release of both amino acids; low concentrations enhanced release, high concentrations depressed release.

6 Bicuculline hydrochloride and picrotoxin both significantly reduced the barbiturate-induced enhancement of K^+-stimulated amino acid release, but did not significantly alter the depression of K^+-stimulated release at higher barbiturate concentrations.

7 Baclofen, either alone (1 μM to 1 mM), or tested against the barbiturates, had no detectable effect.

Introduction

Although anaesthetics generally affect synaptic transmission at lower concentrations than nerve trunk conduction (Larrabee & Posternak, 1952), no simple relationship between anaesthetic action and any single aspect of transmission has been consistently demonstrated for all anaesthetics at any particular synapse. Indeed, anaesthetics have been shown to alter transmitter release (Cutler, Markowitz & Dudzinski, 1974; Cutler & Young, 1979; Minchin, 1981), to affect mitochondrial metabolism and calcium sequestration (Krnjević, 1974), to alter transmitter compartmentation and turnover (Cremer & Lucas, 1971; Cheng, Naruse & Brunner, 1978) to depress postsynaptic responses to excitatory transmitters (Barker & Gainer, 1973; Barker, 1975) to enhance pre- (Eccles, Schmidt & Willis, 1963) and postsynaptic inhibition (Nicoll, Eccles, Oshima & Rubia, 1975) and to interact with γ-aminobutyric acid (GABA) receptors/ionophores (Ransom & Barker, 1975; Macdonald & Barker, 1979). However, whether any or all of these phenomena are causally related to anaesthesia remains uncertain.

It is likely that some sites within the CNS are particularly sensitive to anaesthetics and Angel (1977), and Angel & Unwin (1970), have suggested that transmission through the thalamus is more susceptible to the action of anaesthetic agents than other synapses on the dorsal column-lemnisco-thalamic sensory pathway. Therefore, this site would appear to be a suitable one for biochemical investigation. Since GABA is a possible inhibitory transmitter (Andersen & Curtis, 1964; Duggan & McLennan, 1971; Curtis & Johnston, 1974) and L-glutamate and/or L-aspartate are possible excitatory transmitters (Curtis & Johnston, 1974) within the thalamus, these substances were chosen for examination of possible presynaptic effects of intravenous anaesthetic agents in thalamic slices.

Some of the results have been communicated in preliminary form (Kendall & Minchin, 1980).

Methods

Adult male, Wistar rats (Sheffield strain; 100–200 g) were decapitated and their brains rapidly removed and chilled. Each brain was placed on a McIlwain tissue chopper, where, with ventral surface upwards, coronal slices were taken at intervals of 2, 2 and 1 mm beginning at the caudal margin of the optic chiasma. The thalamus was dissected from each slice as shown in Figure 1 and weighed. Slabs of thalamus (mean

[1]Present address: MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

0007-1188/82/010219-09 $01.00

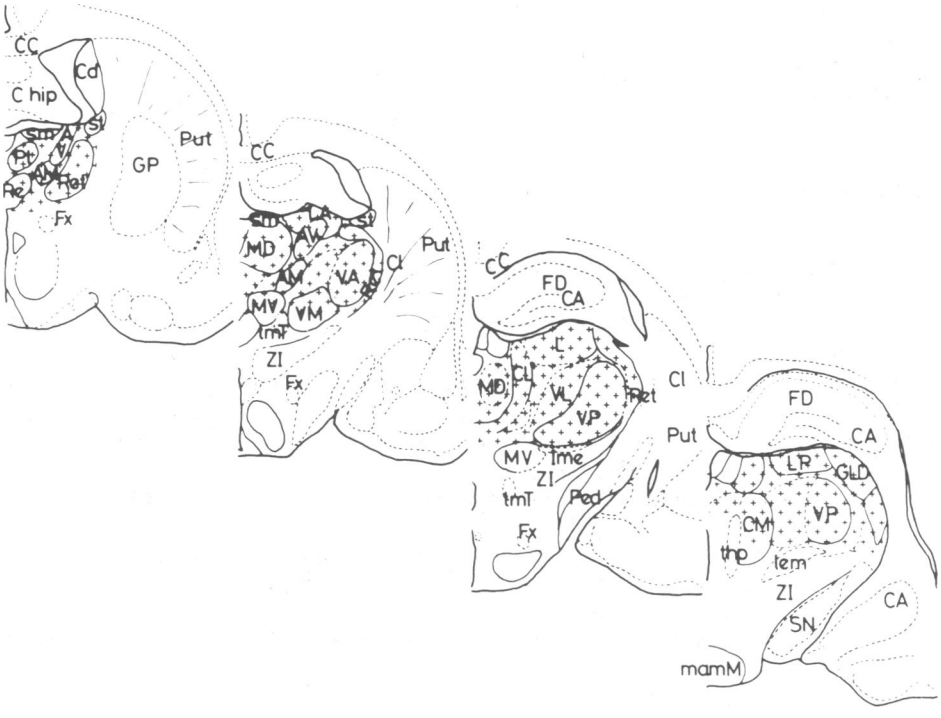

Figure 1 The figure shows four brain sections of the rat redrawn from *Atlas Stereotaxique* constructed by Albe-Fessard, Stutsinsky & Liboubau (1966). The shaded areas demonstrate the thalamic areas removed in dissection and retained for uptake and release experiments. All sections are separate from each subsequent section by 1 mm. The first section (Ant. 7) was taken from a position just posterior to the caudal margin of the optic chiasma. For key to abbreviations and further description of co-ordinates, see Albe-Fessard *et al.* (1966).

weight = 215 ± 5.3 mg s.e.mean, $n = 27$) were then cut at 0.1 mm intervals in two directions at 45° to produce prism-shaped slices (0.1 × 0.1 × approx. 2 mm).

Uptake experiments

Uptake experiments were carried out essentially as described by Iversen & Neal (1968), with the following modifications. Tissue slices were suspended at 1 mg/ml in 10 ml ice-cold incubation medium after gassing with oxygen. After 10 min pre-incubation at 37°C (except blanks which were kept at 0°C throughout the experiment), 50 µl of a solution containing [^{3}H]-D-aspartate and [^{14}C]-GABA was added to each flask to give a final concentration of 0.25 nM and 11 nM respectively. After a further incubation of 10 min, slices were collected by rapid filtration on to a Whatman No. 1 filter paper disc (2.5 cm diam.), washed through with 5 ml of ice-cold incubation medium and transferred to scintillation vials. The filters were soaked in 1.5 ml of distilled water for at least 20 min and 10 ml of scintillation mixture added to each vial. Radioactivity was estimated by liquid scintillation spectrometry.

Release experiments

Tissue slices were suspended in 5 ml ice-cold incubation medium (5 mg/ml) and pre-incubated for 10 min at 37°C; 50 µl portions of a solution containing [^{3}H]-D-aspartate and [^{14}C]-GABA were added to give a final concentration of 22 nM and 0.45 µM respectively and incubation continued for a further 15 min. Slices were then recovered by filtration on to glass fibre filter discs (Whatman GF/A, 2.5 cm diam.) and washed with 5 ml of warm oxygenated medium. Each filter was rapidly transferred to a closed Sartorius filter holder (Sartorius-Membrane filter GmbH, Göttingen, W. Germany) and superfused with warm oxygenated perfusion medium at 0.5 ml/min. A preliminary perfusion period of 30 min was allowed to wash out any remaining extracellular material and to achieve a stable, basal rate of efflux of the two labelled amino acids from the tissue slices.

Fractions were collected in separate scintillation

vials every 3 min. Each control filter was exposed to unmodified perfusion medium for the first six collection periods to allow determination of the basal efflux rate. A period of 9 min followed during which the slices were exposed to perfusion medium containing K^+ 40 mM or protoveratrine A 10 μM to depolarize the tissue. During the remaining 15 min, slices were exposed to normal perfusion medium again.

With this procedure, three filters were used as controls in each experiment whilst various drugs and ions were tested on the three remaining filters. In the latter instance, drugs and ions were present for 6 min before and during exposure to depolarizing agents.

Finally, the tissue slices were removed following the termination of perfusion, placed in scintillation vials and soaked in 1.5 ml of distilled water for at least 20 min to extract the labelled amino acids. After the addition of 10 ml of scintillation mixture to each vial, radioactivity was counted by liquid scintillation spectrometry.

Analysis of release experiments

The efflux rate constant was calculated for each wash as described previously (Minchin, 1981). The increase in the efflux rate constant found during exposure to depolarizing agents was expressed as the percentage increase over the mean basal efflux rate constant. Experimental values were then assessed as a percentage of control and differences assessed by Student's *t* test.

Materials

Incubation and perfusion medium consisted of Krebs phosphate solution containing amino-oxyacetic acid 10 μM to inhibit GABA metabolism by 4-aminobutyrate-2-oxoglutarate aminotransferase (E.C. 2.6.1.19.).

In all experiments, labelled D-aspartate was used in preference to L-aspartate because the former is transported by the same carrier system as L-aspartate, and the uptake of D-aspartate is into the same osmotically sensitive particles as those which accumulate L-aspartate, but D-aspartate does not undergo rapid metabolism (Davies & Johnston, 1976). Furthermore, slices and homogenates of rat brain transport L-aspartate, and therefore D-aspartate, by the same high and low affinity systems that transport L-glutamate (Balcar & Johnston, 1972).

Krebs phosphate solution had the following composition (mM): NaCl 118, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.2, D-glucose 5.6 and NaH_2PO_4 buffer 15, pH 7.4. In some experiments, $CaCl_2$ was omitted and $MgSO_4$ either left at 1.2 mM or increased to 10 mM. For high potassium concentrations, KCl replaced some of the NaCl to give a final concentration of 40 mM K^+. The scintillation mixture was composed of 0.3% diphenyloxazole in a toluene: Triton X-100 (2:1 v/v) mixture. The following radiochemicals were purchased from the Radiochemical Centre, Amersham, Bucks; D-[2,3,-^{3}H] aspartic acid (sp. act. 18 Ci/mmol), and 4-amino-*n*-[U-^{14}C] butyric acid (sp. act. 226 mCi/mmol). Drugs used in this study were as follows: thiopentone (Intraval): methohexitone (Brietal), ketamine (Parke Davis & Co.) urethane (Ethyl Carbamate; May and Baker Ltd, Dagenham), bicuculline, picrotoxin, protoveratrine A, amino-oxyacetic acid (all from Sigma Laboratories), (±)-baclofen (Lioresal; gift from Ciba Geigy) and compound D-600 (gift from Knoll A.G., Ludwigshafen am Rhein, W. Germany).

Results

Effect of anaesthetics on uptake

Control uptake (d min^{-1} mg^{-1} wet wt.) was 744 ± 5 (s.e.mean, $n = 12$) and 411 ± 6 ($n = 12$) for [^{3}H]-D-aspartate and [^{14}C]-GABA, respectively.

Ketamine (100 μM), urethane (15 mM), thiopentone (1 μM) and methohexitone (30 μM) had no significant effect on uptake of either amino acid. However, 1 mM methohexitone increased uptake of [^{3}H]-D-aspartate and [^{14}C]-GABA to 150 ± 6 ($n = 8$, $P < 0.005$) and 125 ± 6 ($n = 8$, $P < 0.005$) percent of controls respectively.

Spontaneous release

After achieving a stable rate of efflux, none of the anaesthetics tested, nor depletion of Ca^{2+} in the perfusing medium had any effect on spontaneous release of either amino acid, with the exception of compound D-600, a calcium antagonist which interferes with transmembrane calcium fluxes (Kohlhardt, Bauer, Krause & Fleckenstein, 1972) which depressed spontaneous release by 17% for both D-aspartate and GABA

Potassium-stimulated release

Figure 2 shows the time course of GABA (Figure 2a) and D-aspartate (Figure 2b) efflux in the presence and absence of calcium in the perfusing medium. The quantitative effects of various low calcium-containing media on the efflux of GABA and D-aspartate are given in Table 1.

Protoveratrine A-stimulated efflux

Protoveratrine enhanced the efflux of both GABA

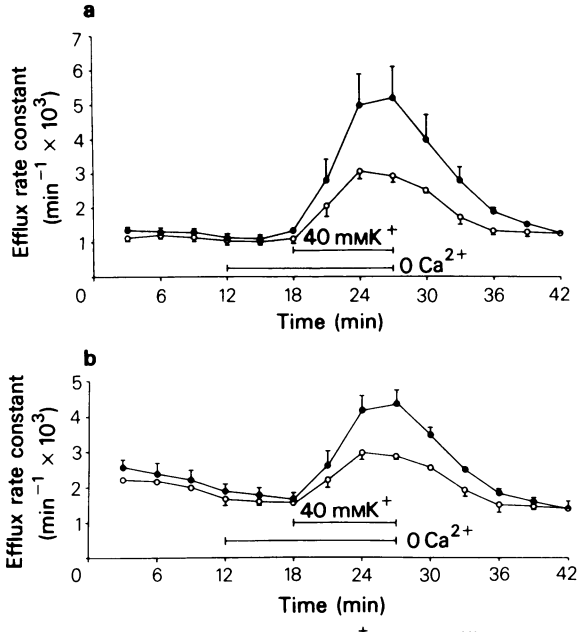

Figure 2 The effect of 40 mM K^+ on the efflux rate constant for (a) [^{14}C]-GABA and (b) [^{3}H]-D-aspartate in the presence (●) and absence (○) of Ca^{2+}. Time of exposure to Ca^{2+}-free medium and elevated K^+ is indicated by the horizontal lines. Each point is the mean of 3 experiments. Vertical bars indicate s.e.mean.

and D-aspartate; removal of Ca^{2+} from the perfusing medium potentiated this effect (Table 1).

Thiopentone 300 μM reduced protoveratrine A-stimulated release to 50±2% ($n=3$, $P<0.01$) of control for [^{3}H]-D-aspartate and to 53±6% ($n=3$, $P<0.01$) of control for [^{14}C]-GABA.

The effect of anaesthetics on K^+-stimulated release

Figures 3 and 4 show the dose-response curves for thiopentone and methohexitone respectively on K^+-stimulated release of [^{3}H]-D-aspartate and [^{14}C]-GABA. Both barbiturates produced a biphasic dose-response, low concentrations enhancing release and high concentrations depressing release. The enhancement of GABA release was slightly more marked than that of D-aspartate. Ketamine (1 μM and 100 μM) and urethane (1 mM and 15 mM) had no significant effect upon release of either amino acid. The biphasic dose-response to the two barbiturates was examined further in an attempt to characterize each phase of this interaction.

The effects of zero calcium (10 mM Mg^{2+}) on barbiturate interactions

In the absence of calcium (10 mM Mg^{2+}), thiopentone 700 μM significantly reduced the K^+-stimulated release of [^{3}H]-D-aspartate to 57±10 ($n=3$,

Table 1 The effect of low calcium-containing media on depolarization-induced release of γ-aminobutyric acid (GABA) and D-aspartate

K^+-stimulated release		
Alteration to medium	*% increase in fractional efflux rate constant*	
	GABA	D-aspartate
None, pooled controls	390±20 (66)	160±10 (63)
	% inhibition of release	
Omission of Ca^{2+}	48± 7 (3)†	39± 6 (3)†
10 mM Mg^{2+} (0 mM Ca^{2+})	56± 6 (5)††	57±11 (5)††
Compound D-600 (0.1 mM)	55±12 (3)††	48±12 (3)††
Protoveratrine-stimulated release		
Alteration to medium	*% increase in fractional efflux rate constant*	
	GABA	D-aspartate
None, controls	130±10 (3)	120±10 (3)
	% of control release	
Omission of Ca^{2+}	140±5 (3)†	181±10 (3)†

†$P<0.05$; ††$P<0.001$ 2-tailed t test, compared with control.
Figures represent the mean ± s.e.mean of the number of experiments in parentheses. Release evoked in low calcium media was compared to controls run in parallel. Depolarization was achieved by exposing the slices to either 40 mM K^+ or 10 μM protoveratrine A for 9 min.

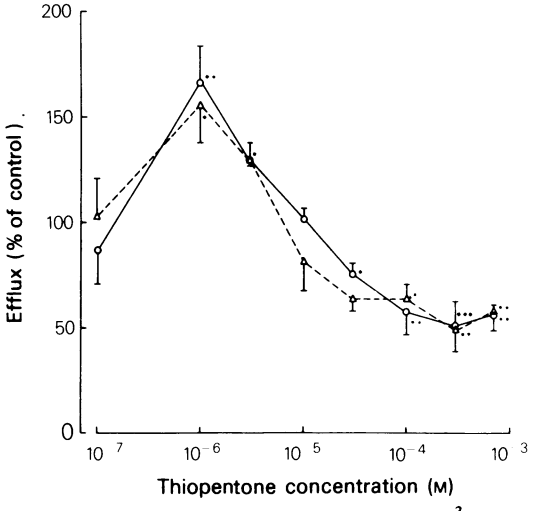

Figure 3 The effect of thiopentone on efflux of [^{3}H]-D-aspartate and [^{14}C]-GABA. The figure shows the dose-response curves for thiopentone on the K^+-stimulated release of the two amino acids. Each point represents the mean of 3–9 experiments; s.e.mean is indicated by vertical bars. Each value represents the mean change in K^+-stimulated release of radioactivity in the presence of thiopentone, expressed as a percentage of the control value: (○) = values for GABA release; (△) = values for D-aspartate release. Statistically significant differences compared with controls are shown as follows: $*P<0.05$; $**P<0.01$; $***P<0.001$.

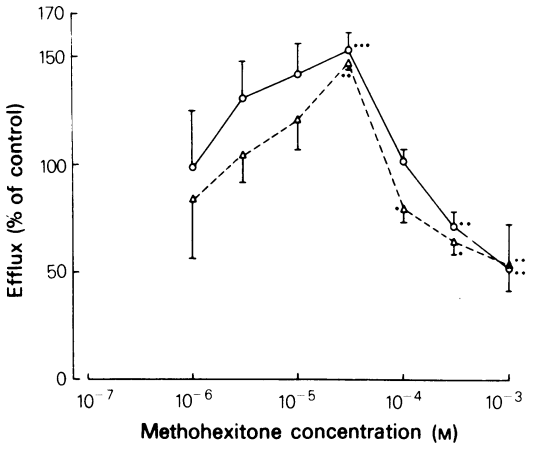

Figure 4 The effect of methohexitone on efflux of [^{3}H]-D-aspartate and [^{14}C]-GABA. Dose-response curves for methohexitone on the K^+-stimulated release of the two amino acids are shown. Each point represents the mean of 3–9 experiments; s.e.mean is indicated by vertical bars. Each value represents the mean change in K^+-stimulated release of radioactivity in the presence of methohexitone, expressed as a percentage of control values: (○) = values for GABA release; (△) = values for D-aspartate release. Statistically significant differences compared with controls are shown as follows: $*P<0.05$; $**P<0.01$; $***P<0.001$.

$P<0.05$) percent of control, whereas the efflux of GABA was not significantly altered. When slices were exposed to thiopentone 1 μM in the absence of calcium (with 10 mM Mg^{2+}), no significant change in release of either amino acid was seen. In these experiments, controls were depolarized in the absence of Ca^{2+}.

The effect of bicuculline, picrotoxin and baclofen on barbiturate interactions

Bicuculline hydrochloride (BHC; 100 μM) and picrotoxin (100 μM) were used in an attempt to antagonize either one of the phases of the barbiturate interaction. The effect of BHC and picrotoxin on the inhibition and enhancement of putative transmitter release by barbiturates is shown in Table 2. Both convulsants significantly reduced the enhancement of release caused by the barbiturates, without affecting the inhibition of release induced by higher barbiturate concentrations. Baclofen (1 μM – 1 mM) had no effect on the release of either amino acid when tested alone or in combination with high and low concentrations of barbiturate.

Discussion

The present investigation was primarily designed to determine the effect of barbiturate and other anaesthetic agents on two aspects of synaptic transmission for an inhibitory transmitter, GABA, and an excitatory transmitter candidate, aspartic acid, in thalamic tissue and to contrast the findings with those described previously in the cerebral cortex (Minchin, 1981). The concentrations of anaesthetics used cover a range around that expected in the brain during anaesthesia (see Minchin, 1981).

Uptake of amino acids

Previous studies have usually demonstrated either small decremental changes, or no significant change at all, in transmitter uptake into rat cortical slices (Cutler & Dudzinski, 1974; Minchin, 1981), hippocampal slices (Jessell & Richards, 1977), hippocampal synaptosomal preparations (Peck, Miller & Lester, 1976) and into olfactory cortex slices (Collins, 1980) as a result of exposure to anaesthetics. In the present study, only methohexitone (1 mM) significantly altered uptake of both amino acids into thalamic tissue. However, this concentration is considerably in excess of that expected in the brain

Table 2 The effect of (a) bicuculline hydrochloride (BHC) and (b) picrotoxin on the inhibition and enhancement of amino acid efflux by anaesthetics

	(a) K^+-stimulated release in the presence of BHC (100 μM) (% of control)	
Anaesthetic	*[^{3}H]-D-aspartate*	*[^{14}C]-GABA*
Methohexitone (1 mM)	118 ± 15 $n = 5$ NS	108 ± 8 $n = 5$ NS
Methohexitone (30 μM)	81 ± 10 $n = 5$ $P < 0.05$	77 ± 7 $n = 5$ $P < 0.05$
Thiopentone (1 μM)	62 ± 7 $n = 5$ $P < 0.005$	83 ± 8 $n = 5$ $P < 0.05$
	(b) K^+-stimulated release in the presence of picrotoxin (100 μM) (% of control)	
Anaesthetic	*[^{3}H]-D-aspartate*	*[^{14}C]-GABA*
Methohexitone (1 mM)	83 ± 6 $n = 6$ NS	84 ± 8 $n = 6$ NS
Methohexitone (30 μM)	68 ± 15 $n = 5$ $P < 0.05$	82 ± 7 $n = 5$ $P < 0.05$

Experiments were carried out as described in Methods. All values are expressed as the mean percentage change in K^+-stimulated release in the presence of both the convulsant and the anaesthetic as compared with controls (anaesthetic present but no convulsant) ± s.e.mean. Number of experiments and statistical significance are given in each case.

during anaesthesia (Minchin, 1981) and so this effect is unlikely to be causally related to anaesthetic action.

Amino acid release and barbiturate inhibition

It is likely that more than half of the GABA released from thalamic tissue in response to potassium depolarization was derived from nerve terminals since efflux was mostly calcium-dependent. This is in agreement with the results of previous studies (Cutler *et al.*, 1974; Cutler & Young, 1979; Minchin, 1981). Furthermore, the potassium-stimulated release of GABA was not significantly depressed by thiopentone 700 μM in the absence of calcium, a phenomenon also seen in cerebral cortex slices (Minchin, 1981). This would suggest that high concentrations of the barbiturates depressed release by an action on nerve terminals. This conclusion is supported by results with protoveratrine A, an alkaloid thought to depolarize specifically, and thereby evoke release from, neuronal terminals but not glial elements (Minchin, 1980). Since neither BHC nor picrotoxin altered the barbiturate-induced depression of K^+-stimulated GABA release, it does not appear to be related to an action at a GABA receptor.

Similar considerations apply to D-aspartate release. It was largely calcium-dependent, which is in accord with previous suggestions of a neurotransmitter function for this amino acid (Davies & Johnston, 1976; Collins, 1979; Malthe-Sørrensen, Skrede & Fonnum, 1979) and barbiturate-induced depression of release was not significantly affected by either BHC or picrotoxin. However, thiopentone 700 μM depressed D-aspartate release in the presence of Mg^{2+} 10 mM, suggesting some depression of non-neuronal release. This contrasts with findings in cerebral cortex slices where Mg^{2+} 10 mM abolished the ability of thiopentone to inhibit D-aspartate release (Minchin, 1981).

In Ca^{2+}-containing media, the inhibition of K^+-stimulated amino acid release by barbiturates was similar to that described in cortical slices (Minchin, 1981), although unlike the latter, it did not exceed 50%. As in cortical slices, methohexitone was less potent than thiopentone, although as an anaesthetic it is more potent (Minchin, 1981) and inhibition of release by methohexitone did not occur at anaesthetic concentrations. Therefore whilst inhibition of release in thalamic tissue, particularly of excitatory amino acids, by thiopentone may be involved in anaesthesia the same cannot be said for methohexitone.

Barbiturate-enhanced amino acid release

In the present study, low concentrations of methohexitone and thiopentone increased the potassium-stimulated release of both GABA and D-aspartate, probably by an action on nerve terminals since thiopentone-induced enhancement was absent in Ca^{2+}-free medium. GABA release was consistently, but not significantly enhanced to a greater extent than that of D-aspartate. Furthermore, the enhancement of release was antagonized by BHC and picrotoxin, possibly implicating a presynaptic GABA receptor as the site of action of the barbiturates at these concentrations. Regulation of GABA and aspartate release by presynaptic GABA receptors in other CNS regions has been demonstrated previously although hitherto agonist action at such receptors has resulted in inhibition of release rather than potentiation (Mitchell & Martin, 1978; Snodgrass, 1978; Collins, 1980). Although the present results suggest that GABA receptor activation leads to an increase in evoked release, further characterization of the mechanisms involved is necessary. The possibility that dendritic release is involved (see below) complicates the issue since regulation of such release may well be pharmacologically quite different from regulation of axon terminal release.

In an attempt to characterize further the regulation of GABA and D-aspartate release, the antispastic drug, baclofen, was tested both alone and with high and low concentrations of barbiturate. Baclofen has been shown to decrease excitatory transmission and release (Pierau & Zimmerman, 1973; Pierau, Matheson & Wurster, 1975; Potashner, 1979) and Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull (1980) have suggested that it decreases transmitter release by a presynaptic action at a novel, bicuculline-insensitive GABA receptor. Baclofen was therefore tested in the present system since the biphasic response to each barbiturate may have reflected a dual action at two different GABA receptors, a bicuculline-sensitive receptor and a baclofen-sensitive receptor. However, at no concentration, either with or without barbiturates present, did baclofen have any effect.

To date, three *in vitro* preparations have been shown to have a biphasic dose-response to barbiturate application. In the isolated retina of the rabbit, spontaneous GABA release was depressed by pentobarbitone 1 mM, whereas pentobarbitone 10 μM initially enhanced release (Bauer, 1979). In olfactory cortex slices, electrically evoked synaptic GABA release was significantly enhanced by administration of 100 μM pentobarbitone but depressed at 1 mM pentobarbitone (Collins, 1980). A similar biphasic dose-response to both methohexitone and thiopentone was seen in the present study. All three CNS regions have a very complex synaptic arrangement and both the retina and thalamus contain cells whose dendrites contain synaptic vesicles and other organelles usually present in axon terminal boutons. Such dendrites make dendro-dendritic contacts similar in organization to a conventional synapse (thalamus: Ralston & Herman, 1969; Harding, 1971; Leiberman & Webster, 1972; retina: Dowling & Boycott, 1966; Dowling, Brown & Major, 1966). Furthermore, Leiberman & Webster (1972) have shown that presynaptic dendrites in the thalamus give rise to boutons packed with synaptic vesicles, which, because of their flattened disc-like shape, may contain inhibitory transmitter molecules (Uchizono, 1965). These dendritic boutons constitute one of the principal classes of synaptic endings in the thalamic glomeruli (Leiberman & Webster, 1972), but whether such structures exist in the olfactory cortex is not known. The relevance of such ultrastructural findings in relation to barbiturate action is at present unclear, but the obvious enhancement of GABA release in the olfactory cortex, retina and thalamus, not shown in other brain areas (with the exception of that shown by Cutler & Dudzinski (1974) at high barbiturate concentrations in rat cortical slices) deserves further investigation.

A final consideration relates to the recent work of Huang & Barker (1980), who have demonstrated that the different stereoisomers of pentobarbitone appear to have opposite effects on embryonic spinal cord cells. Since both methohexitone and thiopentone have asymmetric carbon atoms, it is possible that only one stereoisomer has a GABA receptor activating effect. If this were the case, the biphasic dose-response curves may represent activation of a GABA receptor by low concentrations of one stereoisomer, but the other stereoisomer, at higher concentrations, may overshadow such receptor activation, perhaps by a non-specific membrane stabilization, so reducing ion fluxes and decreasing transmitter release.

In comparing the effects of barbiturates on cortical and thalamic slices, the enhancement of transmitter release seen in the latter but not the former (Minchin, 1981) is a clear distinction and it is tempting to relate this to the depression of transmission of sensory information which occurs in the thalamus but not in the cortex during anaesthesia (Angel & Unwin, 1970). However, only methohexitone enhanced release at anaesthetic concentrations and the non-barbiturates tested in the present study had no effect at all, despite the fact that they depress thalamic transmission (Angel & Unwin, 1970; Angel, personal communication). Therefore, not all anaesthetics perform their physiological function in the thalamus by enhancing amino acid transmitter release, although methohexitone might and thiopentone may

during the earliest stage of anaesthesia when its concentration in the brain would be low.

The enhanced release of both GABA and D-aspartate caused by barbiturates may originate from descending cortico-thalamic fibres since decortication abolishes it, leaving the inhibition of release seen at higher barbiturate concentrations unaffected (Kendall, Minchin & Angel, unpublished observation). It is possible, therefore, that barbiturates enhance not only a direct descending inhibition in the thalamus but also the effects of a descending excitatory pathway that in turn may activate inhibitory interneurones and give rise to release of GABA, perhaps by a dendritic release mechanism.

In conclusion, the data presented here suggest a presynaptic mechanism for some barbiturate-induced anaesthetic effects which does not seem to apply to non-barbiturates and involves a change in the balance between ascending and descending excitation and inhibition in favour of inhibition. This, combined with the postsynaptic effects of barbiturates described by others, may in some cases underlie the block of sensory transmission which is associated with the state of anaesthesia.

This work was supported by the MRC. We wish to thank Dr A. Angel for his helpful advice and criticism. Reprint requests to M.C.W.M. in Oxford.

References

ALBE-FESSARD, D., STUTSINSKY, F. & LIBOUBAU, S. (1966). *Atlas Stereotaxique du Diencaphale du Rat blanc.* Edition du centre national de la recherche scientifique.

ANDERSEN, P. & CURTIS, D.R. (1964). The pharmacology of the synaptic and acetylcholine-induced excitation of ventrobasal thalamic neurones. *Acta. Physiol. scand.*, **61,** 100–200.

ANGEL, A. (1977). Processing of sensory information. *Progr. Neurobiol.*, **9,** 1–122.

ANGEL, A & UNWIN, J. (1970). Effect of urethane on transmission along dorsal column sensory pathway in rat. *J. Physiol.*, **208,** 32P.

BALCAR, V.J. & JOHNSTON, G.A.R. (1972). The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. *J. Neurochem.*, **19,** 2657–2666.

BARKER, J.L. (1975). CNS depressants: effects on post synaptic pharmacology. *Brain Res.*, **92,** 35–55.

BARKER, J.L. & GAINER, H. (1973). Pentobarbital: selective depression of excitatory post synaptic potentials. *Science,* **182,** 720–722.

BAUER, B. (1979). Effect of pentobarbitone on spontaneous efflux of gamma-amino acids from rabbit retina. *Brain Res.*, **163,** 307–317.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDLEMISS, D.N., SHAW, J. & TURNBULL, M. (1980). (−) Baclofen decreases neurotransmitter release in mammalian CNS by an action at a novel GABA receptor. *Nature, Lond.*, **283,** 92–94.

CHENG, S.-C., NARUSE, H. & BRUNNER, E.A. (1978). Effects of sodium thiopental on the tricarboxylic acid cycle metabolism in mouse brain: CO_2 fixation and metabolic compartmentation. *J. Neurochem.*, **30,** 1591–1593.

COLLINS, G.G.S. (1979). Evidence of a neurotransmitter role for aspartate and γ-aminobutyric acid in the rat olfactory cortex. *J. Physiol.*, **291,** 51–60.

COLLINS, G.G.S. (1980). Release of endogenous amino acid neurotransmitter candidates from rat olfactory cortex: possible regulatory mechanisms and the effects of pentobarbitone. *Brain Res.*, **190,** 517–528.

CREMER, J.E. & LUCAS, H.M. (1971). Sodium pentobarbitone and metabolic compartments in rat brain. *Brain Res.*, **35,** 619–621.

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, **69,** 97–188.

CUTLER, R.W.P. & DUDZINSKI, D.S. (1974). Effect of pentobarbital on uptake and release of [^{3}H]-GABA and [^{14}C]-glutamate by brain slices. *Brain Res.*, **67,** 546–548.

CUTLER, R.W.P., MARKOWITZ, D. & DUDZINSKI, D.S. (1974). The effects of barbiturates on [^{3}H]-GABA transport in rat cerebral cortex slices. *Brain Res.*, **81,** 189–197.

CUTLER, R.W.P. & YOUNG, J. (1979). Effect of barbiturates on release of endogenous amino acids from rat cortex slices. *Neurochem. Res.*, **4,** 319–329.

DAVIES, L.P. & JOHNSTON, G.A.R. (1976). Uptake and release of D- and L-aspartate by rat brain slices. *J. Neurochem.*, **26,** 1007–1014.

DOWLING, J.E. & BOYCOTT, B.B. (1966). Organisation of the primate retina: electron microscopy. *Proc. R. Soc. B.*, **166,** 80–111.

DOWLING, J.E., BROWN, J.E. & MAJOR, D. (1966). Synapses of horizontal cells in rabbit and cat retinas. *Science,* **153,** 1639–1641.

DUGGAN, A.W. & McLENNAN, H. (1971). Bicuculline and inhibition in the thalamus. *Brain Res.*, **25,** 188–191.

ECCLES, J.C., SCHMIDT, R. & WILLIS, W.D. (1963). Pharmacological studies on pre-synaptic inhibition. *J. Physiol.*, **168,** 500–530.

HARDING, B.N. (1971). Dendrodendritic synapses, including reciprocal synapses in the ventrolateral nucleus of the monkey thalamus. *Brain Res.*, **34,** 181–185.

HUANG, L-Y.M. & BARKER, J.L. (1980). Pentobarbital stereospecific actions of (+) and (−) isomers revealed on cultured mammalian neurones. *Science,* **207,** 195–197.

IVERSEN, L.L. & NEAL, M.J. (1968). The uptake of [^{3}H]-GABA by slices of rat cerebral cortex. *J. Neurochem.*, **15,** 1141–1149.

JESSELL, T.M. & RICHARDS, C.D. (1977). Barbiturate

potentiation of hippocamal i.p.s.p.'s is not mediated by blockade of GABA uptake. *J. Physiol.*, **269,** 42–43P.

KENDALL, T.J.G. & MINCHIN, M.C.W. (1980). The effect of thiopentone and methohexitone on [^{14}C]-GABA and [^{3}H]-D-aspartate release from rat thalamic slices. *Br. J. pharmac.*, **70,** 134–135P.

KOHLHARDT, M., BAUER, B., KRAUSE, H. & FLECKENSTEIN, A. (1972). New selective inhibitors of the transmembrane calcium conductivity in mammalian myocardial fibres. Studies with the voltage clamp technique. *Experientia*, **28,** 288–289.

KRNJEVIĆ, K. (1974). Central actions of general anaesthetics. In: *Molecular Mechanisms in General Anaesthesia*, ed. Halsey, M.J., Millar, R.A. & Sutton, J.A. pp. 65–89. London: Churchill Livingstone.

LARRABEE, M.G. & POSTERNAK, J.M. (1952). Selective action of anaesthetics on synapses in mammalian sympathetic ganglia. *J. Neurophysiol.*, **15,** 91–114.

LEIBERMAN, A.R. & WEBSTER, K.E. (1972). Presynaptic dendrites and a distinctive class of synaptic vesicles in the rat dorsal lateral geniculate nucleus. *Brain Res.*, **42,** 196–200.

MACDONALD, R.C. & BARKER, J.L. (1979). Anticonvulsant and anaesthetic barbiturates: different postsynaptic actions in cultured mammalian neurones. *Neurology*, **29,** 432–447.

MALTHE-SØRENSSEN, D., SKREDE, K.K. & FONNUM, F. (1979). Calcium-dependent release of [^{3}H]-D-aspartate evoked by selective electrical stimulation of excitatory afferent fibres to hippocamal pyramidal cells. *Neurosci.*, **4,** 1255–1263.

MINCHIN, M.C.W. (1980). Veratrum alkaloids as transmitter-releasing agents. *J. Neurosci. Methods*, **2,** 111–121.

MINCHIN, M.C.W. (1981). The effect of anaesthetics on the uptake and release of γ-aminobutyrate and D-aspartate in rat brain slices. *Br. J. Pharmac.*, **73,** 681–689.

MITCHELL, P.R. & MARTIN, I.L. (1978). Is GABA release modulated by presynaptic receptors? *Nature, Lond.*, **274,** 904–905.

NICOLL, R.A., ECCLES, J.C., OSHIMA, T. & RUBIA, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. *Nature, Lond.*, **258,** 625–627.

PECK, E.J., MILLER, A.L. & LESTER, B.R. (1976). Pentobarbital and synaptic high-affinity receptive sites for gamma-aminobutyric acid. *Brain Res. Bull.*, **1,** 595–597.

PIERAU, F-K. & ZIMMERMAN, P. (1973). Action of a GABA derivative on post-synaptic potentials and membrane properties of cat spinal motoneurones. *Brain Res.*, **54,** 376–380.

PIERAU, F-K., MATHESON, G.K. & WURSTER, R.D. (1975). Pre-synaptic action of β-(4-chlorophenyl) GABA. *Exp. Neurol.*, **48,** 343–351.

POTASHNER, S.J. (1979). Baclofen: Effects on amino acid release and metabolism in slices of guinea pig cortex. *J. Neurochem.*, **32,** 103–109.

RALSTON, H.J. & HERMAN, M.M. (1969). The fine structure of neurones and synapses in the ventrobasal thalamus of the cat. *Brain Res.*, **14,** 77–97.

RANSOM, B.R. & BARKER, J.L. (1975). Pentobarbital modulates transmitter effects on mouse spinal neurones grown in tissue culture. *Nature, Lond.*, **254,** 703–705.

SNODGRASS, S.R. (1978). Use of ^{3}H-muscimol for GABA receptor studies. *Nature*, **273,** 392–394.

UCHIZONO, K. (1965). Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. *Nature, Lond.*, **207,** 642–643.

(Received July 15, 1981.
Revised September 21, 1981.)