The action of pentobarbitone on stimulus-secretion coupling in adrenal chromaffin cells

G. Pocock & C.D. Richards

Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF

- 1 The action of pentobarbitone on stimulus-secretion coupling was studied in bovine isolated adrenal medullary cells.
- 2 Pentobarbitone inhibited catecholamine release evoked by 500 μM carbachol with half maximal inhibition (IC_{50}) around 50 μM . It also inhibited catecholamine release induced by depolarization with 77 mM potassium (IC_{50} 100 μM). These effects of pentobarbitone were observed with concentrations that lie within the range encountered during general anaesthesia.
- 3 Evoked secretion required the presence of calcium in the extracellular medium and was associated with an influx of Ca^{2+} through voltage-sensitive channels. Pentobarbitone inhibited ^{45}Ca influx in response to both carbachol (IC_{50} 50 μM) and K^{+} -depolarization (IC_{50} 150 μM).
- 4 The action of pentobarbitone on the relationship between intracellular free Ca and exocytosis was examined using electroporpermabilised cells which were suspended in solutions containing a range of concentrations of ionised calcium between 10^{-8} and 10^{-4} M. Catecholamine secretion was measured in the presence of 0, 50, 200 or 500 μM pentobarbitone. The anaesthetic had no effect on the activation of exocytosis by intracellular free calcium.
- 5 When catecholamine secretion in response to potassium or carbachol was modulated by varying extracellular calcium or by adding pentobarbitone to the incubation medium, the amount of catecholamine secretion for a given Ca^{2+} entry was the same.
- 6 Pentobarbitone inhibited the secretion and ^{45}Ca uptake induced by carbachol in a non-competitive manner.
- 7 The secretion evoked by nicotinic agonists was associated with an increase in ^{22}Na influx. Pentobarbitone inhibited this influx with an IC_{50} of 100 μM .
- 8 We concluded that: (a) Pentobarbitone inhibits the catecholamine secretion from bovine adrenal chromaffin cells induced by nicotinic agonists by non-competitive inhibition of the nicotinic receptor. (b) The decrease in Ca influx caused by pentobarbitone accounts fully for the decrease in secretion in response to depolarization with potassium.

Introduction

Barbiturates are known to depress excitatory synaptic transmission in the central nervous system at concentrations similar to those required for the induction and maintenance of anaesthesia. Detailed electrophysiological analysis of their mode of action has shown that the process of chemical transmission itself is affected rather than impulse conduction in the afferent fibres or the electrical properties of the postsynaptic cells (Weakly, 1969; Richards, 1972; 1982; Richards & Strupinski, 1986). This implies that their action is mediated by a disturbance of neurosecretion, a reduction in the sensitivity of the postsynaptic receptors, or both. Weakly (1969) found that pentobarbitone and thiopentone decrease the

quantal content of excitatory postsynaptic potentials (e.p.s.ps) recorded from spinal motoneurons and Potashner *et al.* (1980) have shown that the calcium-dependent stimulus-evoked release of the putative excitatory transmitter glutamate is depressed by pentobarbitone. Although barbiturates have been shown to depress calcium conductance in neurones from mouse dorsal root ganglia (Werz & MacDonald, 1985), their effects on the relationship between calcium movement and secretion are not known.

Within the CNS it has proved difficult to assign particular transmitters to specific pathways which can be studied readily *in vitro*. However, clarification of this kind is an essential prerequisite for any detailed

analysis of the action of anaesthetics on the mechanisms of transmitter release.

The adrenal chromaffin cell offers a suitable experimental model for such analysis as the mechanisms of transmitter secretion are well characterized (Baker & Knight, 1984). In addition, it is already known from studies on whole glands that pentobarbitone inhibits the secretion of catecholamine that is induced by nicotinic agonists and high K^+ (Holmes & Schneider, 1973). Chromaffin cells are derived from embryonic neural crest tissue and are homologous with sympathetic postganglionic neurones. The cells may be isolated in large numbers from bovine adrenal glands and form a relatively homogeneous population which secretes adrenaline and noradrenaline. It is also possible to manipulate their intracellular environment using the technique of electroporomeabilisation (Knight & Baker, 1982). They therefore offer considerable advantages over other possible model systems such as synaptosomes.

Activation of the nicotinic receptors on the surface of the chromaffin cell causes inward movement of Na and possibly Ca ions. The resultant depolarization opens voltage sensitive channels to bring about a rise in free Ca^{2+} which triggers granule exocytosis (Knight & Kesteven, 1983). Exposure of cells to high K^+ bypasses the acetylcholine receptor and elicits secretion by opening the voltage sensitive Ca^{2+} channels directly (Baker & Knight, 1981). We made a detailed examination of the action of pentobarbitone on secretion induced by both pathways and on the relationship between intracellular free Ca^{2+} and exocytosis. We concluded that the inhibitory effect of pentobarbitone on secretion results from inhibition of Ca^{2+} movement through the voltage sensitive Ca^{2+} channel and from inhibition of ion movements through the channels gated by the nicotinic receptor.

Methods

Isolation of chromaffin cells

Bovine adrenal glands were obtained from a local abattoir. They were removed from the animals within 30 min of death and perfused via the adrenal vein with 5–10 ml of ice-cold Locke's solution containing 0.2% (w/v) bovine serum albumin. The glands were then transported to the laboratory on ice.

Chromaffin cells were isolated by enzymatic digestion of thin slices of the adrenal medulla as described previously (Pocock, 1983a). Care was taken to remove any obvious islands of cortical tissue. Approximately 5×10^7 cells could be obtained from each gland and 6–10 glands were used in most experiments. The viability of the cells was assessed on the basis of their ability to exclude trypan blue and on this criterion our

preparations contained at least 95% live cells. Furthermore, more than 95% of the total catecholamine present in suspensions was associated with the cells. The cell suspensions contained up to 40% non-chromaffin cells estimated by neutral red and electron microscopy. This raised the possibility that Ca^{2+} fluxes induced by depolarization with high K^+ (but not by carbachol) might have been due in part to influx into cortical cells (see Results and Discussion).

Measurement of catecholamine secretion and ion fluxes

Basal and evoked secretion of adrenaline and noradrenaline and the associated ion fluxes were measured by centrifuging cell suspensions through a layer of oil (see below). Catecholamine was assayed by the method of Von Euler & Floding (1955) using a Perkin-Elmer fluorescence spectrophotometer. At the concentrations used in this study, pentobarbitone did not interfere with this assay.

To examine the effect of pentobarbitone on ion fluxes and secretion, cell suspensions were incubated at 37°C with varying concentrations of the anaesthetic for 5–10 min. Isotope (^{22}Na or ^{45}Ca) was added and the suspensions were then mixed with equal volumes of either Na-Locke, Na-Locke containing carbachol, or Locke solution in which varying amounts of the Na^+ had been replaced with K^+ . Each solution contained an appropriate concentration of anaesthetic. After various time intervals (see Results), triplicate samples from the cell suspensions were centrifuged (12,000 g for 20–30 s) through a layer of oil consisting of di-n-butyl phthalate and light liquid paraffin (10:1 v/v). Sodium influx was measured in the presence of tetrodotoxin (TTX; 10^{-5} M) to prevent Na^+ movement through the voltage sensitive channel and ouabain (2×10^{-5} M added one minute before the addition of ^{22}Na) to inhibit Na^+ efflux via the sodium pump.

The catecholamine in the supernatant was assayed and expressed as a percentage of that present in the total cell suspension after the cells had been lysed with 0.1% Triton X-100. To determine the amount of isotope present in the cells, the centrifuge tubes were frozen and the end containing the pellet was cut off with a razor blade. This technique permits good separation of cells from extracellular fluid and reduces contamination of the pellet with extracellular isotope to very low levels. In experiments with ^{22}Na , the radioactivity associated with the cells was counted directly in a γ -counter. For ^{45}Ca , the pellets were solubilized in 0.5 ml of 0.1% Triton X-100 (v/v in distilled water) before liquid scintillation counting.

The uptake of ^{22}Na or ^{45}Ca was expressed as mM or $\mu\text{mol l}^{-1}$ of cells. The pellet volume was estimated as follows. The relationship between total catecholamine content and cell number was determined in one sample

for each experiment. Total catecholamine content of all subsequent samples was measured and the pellet volume calculated from this relationship. The volume of a single cell was assumed to be 3.054×10^{-12} l. (see Pocock, 1983b).

Electropermeabilised cells

A suspension of cells was washed in Ca^{2+} -free Locke solution containing 0.4 mM EGTA ($\text{pCa} < 8$) and resuspended in a medium similar to the intracellular fluid (see below). The cells were then subjected to 10 high-voltage electric discharges (2 kV cm^{-1}). This treatment renders the cells permeable to small molecules (Knight & Baker, 1982) without damaging intracellular organelles, and the free Ca is determined by that of the extracellular medium. The cells were then added to media containing pentobarbitone (0–500 μM) and CaCl_2 and EGTA in varying ratios to define free Ca^{2+} at the desired levels (pCa was between 4 and 8). The amount of catecholamine released into the medium after 10 min was measured and expressed as a percentage of the total.

Physiological media

Normal Locke solution (Na-Locke) contained (mM): NaCl 140, KCl 5, MgCl_2 1.8, CaCl_2 1.8, HEPES 15, glucose 5.5. The pH was adjusted to 7.4 with NaOH. For K-Locke the NaCl was replaced on an equimolar basis with KCl.

The composition of the electropermeabilisation medium was (mM): potassium glutamate 140, sodium glutamate 5, Mg-ATP 5.0, MgCl_2 1.8, PIPES 15, EGTA 0.4, glucose 5.5 and the pH adjusted to 7.0 with KOH. For experimental measurements, the level of free Ca^{2+} was adjusted by adding varying amounts of CaCl_2 and EGTA and the final pCa determined by an Orion 93–20 Ca electrode.

Results

The action of pentobarbitone on catecholamine secretion

The catecholamine content of our cell suspensions varied from 44 to 133 nmol per 10^6 cells with a median value around 70 nmol per 10^6 cells. In the absence of pentobarbitone, the amount of catecholamine present in the supernatant was 2–5% of the total and it increased slowly with time (approximately 3% of total catecholamine per hour). Addition of carbachol (10–1000 μM) to the medium resulted in a very rapid increase in the rate of secretion of catecholamine which reached a plateau after 3–5 min. A similar increase in secretion was observed when the cell

suspension was added to Locke solution containing elevated levels of K^+ . Stimulation reached a plateau when carbachol was 200–500 μM and K^+ was 50–77 mM. We therefore chose to stimulate the cells with 500 μM carbachol or 77 mM K^+ in most experiments. The quantity of catecholamine released by the cells in response to either method of stimulation was similar. Evoked secretion was generally 4–8% of total which corresponds to 1.8–5.6 nmol of catecholamine secreted per 10^6 cells and a basal secretion of $3\% \text{ h}^{-1}$ corresponds to 2.1 nmol per 10^6 cells. These

Figure 1 The dose-response relationship for the effect of pentobarbitone on catecholamine (CA) secretion (a) and ^{45}Ca uptake (b) induced by 500 μM carbachol (●) and 77 mM potassium (○). The points shown are the mean of 2–7 determinations and the vertical lines represent s.e.mean

values compare favourably with previously published values for freshly isolated chromaffin cells (Pocock, 1980; Derome *et al.*, 1981).

Addition of pentobarbitone (5–500 μM) to the cell suspensions had no significant effect on basal catecholamine secretion but inhibited secretion stimulated by either 500 μM carbachol or 77 mM K^+ -Locke. The extent of the inhibition was dependent on the concentration of the anaesthetic (Figure 1a). Of the evoked catecholamine secretion induced by 77 mM K^+ about 50–60% could be inhibited by pentobarbitone. The IC_{50} for the anaesthetic-sensitive component was approximately 100 μM . Secretion stimulated by carbachol was more readily inhibited (IC_{50} 50 μM). The inhibition for a given concentration was approximately constant throughout the time course of stimulation (Figure 2) and was independent of the time for which the cells were incubated in the presence of pentobarbitone before stimulation (2–20 min; not shown). Consequently, the duration of the pre-incubation period and the time at which the cell suspensions were sampled were not critical.

Under the conditions of our experiments, isolated chromaffin cells did not show consistent responses to repeated stimulation. However, we were able to demonstrate that cells exposed to pentobarbitone showed normal levels of secretion after the drug had been washed from the cells (see Figure 3 for details).

The action of pentobarbitone on ^{45}Ca influx

The increase in secretion of catecholamine that results from stimulation of the cells with carbachol or high K^+ is associated with an increase in Ca^{2+} influx (Holz *et al.*, 1982). The inhibition of secretion by pentobarbitone was accompanied by a proportionate decrease in ^{45}Ca influx (Figure 1b). This inhibition of ^{45}Ca influx was evident throughout the time course of stimulation (Figure 2). However, pentobarbitone did not affect the resting ^{45}Ca influx.

Pentobarbitone (50 μM) inhibited both the secretion and ^{45}Ca uptake induced by carbachol in a non-competitive manner (Figure 4). A similar displacement of the dose-response curve by 50 μM pentobarbitone was observed for the secretion induced by acetylcholine (not shown), and for the effects of 200 μM pentobarbitone on the catecholamine secretion and ^{45}Ca influx induced by elevated concentrations of K^+ (Figure 5).

Ca influx and catecholamine secretion in response to stimulation are dependent on the extracellular Ca^{2+} concentration. Pentobarbitone inhibited secretion at concentrations of extracellular Ca^{2+} between 0.1 and 5.0 mM. The percentage decrease was similar in all cases (Figure 6), suggesting that pentobarbitone is not a competitive inhibitor of Ca^{2+} influx.

We examined the relationship between ^{45}Ca entry

Figure 2 The action of 50 μM and 200 μM pentobarbitone on the time course of catecholamine (CA) secretion and ^{45}Ca uptake induced by (a) 77 mM potassium and (b) 500 μM carbachol: (○) control responses; responses in presence of 50 μM (◆) and 200 μM (●) pentobarbitone.

Figure 3 Demonstration of the reversibility of the effect of pentobarbitone on catecholamine (CA) secretion and ^{45}Ca uptake. A cell suspension was divided into three and treated with 0, 50 or 200 μM pentobarbitone (Pbt) respectively. The cells exposed to pentobarbitone were then further subdivided, half being washed free of anaesthetic and half resuspended in fresh Locke solution containing the same concentration of anaesthetic. The resulting five groups of cells were then stimulated with either 77 mM K^+ (open columns) or 500 μM carbachol (hatched columns). The three pairs of columns on the left show the effect of 0, 50 and 200 μM pentobarbitone on catecholamine secretion (a) and ^{45}Ca influx (b). The two pairs of columns on the right show that removal of pentobarbitone before stimulation results in near normal responses.

and catecholamine secretion under the following conditions: (1) in the presence of varying concentrations of pentobarbitone (20–500 μM) at constant extracellular calcium (1 mM). (2) In the absence of pentobarbitone, with varying extracellular calcium (0.1–5.0 mM).

For cells stimulated by either potassium or carbachol, the amount of catecholamine secretion for a given Ca^{2+} entry was the same under both sets of conditions. This suggests that pentobarbitone inhibits catecholamine secretion by inhibiting Ca^{2+} influx (Figure 7).

Figure 4 The effect of 50 μM pentobarbitone on the dose-response curve for the action of carbachol (CCh) on catecholamine (CA) secretion (a) and ^{45}Ca uptake (b). (○) Control responses, (●) responses in presence of pentobarbitone.

Pentobarbitone does not interfere with exocytosis

We also examined the action of pentobarbitone on the relationship between intracellular free Ca^{2+} and exocytosis in electroporated cells. These cells were suspended in solutions containing a range of concentrations of Ca^{2+} between 10^{-8} and 10^{-4} M; The amount of catecholamine secreted by the cells in the presence of 0, 50, 200 or 500 μM pentobarbitone was the same. The data for 200 μM pentobarbitone are shown in Figure 8. The anaesthetic therefore had no effect on the process of exocytosis itself.

Effect of pentobarbitone on ^{22}Na influx

The catecholamine secretion induced by carbachol was inhibited by 80% in the presence of 100 μM pentobarbitone. In contrast, the secretion induced by high K^+ was only partially inhibited even by 500 μM pentobarbitone. There is, therefore, an additional effect of the anaesthetic on the nicotinic receptor or on

Figure 5 The effect of 200 μM pentobarbitone on the catecholamine (CA) secretion (a) and ^{45}Ca uptake (b) induced by elevated concentrations of potassium. (○) Control responses, (●) responses in presence of pentobarbitone.

Figure 6 The effect of 200 μM pentobarbitone on catecholamine (CA) secretion induced by 77 mM potassium in the presence of varying amounts of calcium in the bathing fluid. (○) Control responses, (●) responses in presence of pentobarbitone.

Figure 7 The relationship between catecholamine (CA) secretion and calcium uptake during stimulation by 77 mM potassium. (○) show the relationship when external calcium is varied in the absence of pentobarbitone and (●) show the relationship in cell suspensions treated with varying concentrations of pentobarbitone and 1 mM Ca. (The pentobarbitone concentrations used were: 20, 50, 100, 200 and 500 μM and the calcium concentrations were: 0.1, 0.25, 0.5, 1.0, 2.0 and 5.0 mM).

Figure 8 The effect of 200 μM pentobarbitone on the activation of catecholamine (CA) secretion in electroporated chromaffin cells by ionised calcium. At low external calcium concentrations ($p\text{Ca} < 6$) the secretion was about 5% over the 15 min period. Raising ionised calcium levels from 10^{-6} to 10^{-4} ($p\text{Ca} 6-4$) caused an increase in secretion. This pattern was not affected by 200 μM pentobarbitone. (○) Control responses, (●) responses in presence of pentobarbitone.

the events associated with its activation. We measured the effect of pentobarbitone on the influx of ^{22}Na and correlated these measurements with the secretion of catecholamine. The cells were pre-incubated as before in the absence and presence of 50 or 200 μM pentobarbitone.

The cells steadily accumulated ^{22}Na in the absence of stimulation. However, when they were stimulated by carbachol the influx of ^{22}Na was increased for 1–2 min. Pentobarbitone did not affect the basal ^{22}Na influx but inhibited the increase in ^{22}Na influx elicited by carbachol by approximately 40% at 50 μM and by 75% at 200 μM . The IC_{50} for the inhibition of ^{22}Na influx was around 100 μM (Figure 9).

We examined the relationship between ^{22}Na entry and catecholamine secretion evoked by carbachol under the following conditions: (1) in the presence of varying concentrations of pentobarbitone (10–200 μM) with 147 mM sodium in the extracellular medium. (2) In the absence of pentobarbitone with progressive replacement of extracellular sodium by N-methyl glucamine (meglumine).

During stimulation with carbachol, the amount of catecholamine secreted following a given entry of ^{22}Na was lower when secretion was inhibited by pentobarbitone than when secretion was inhibited by reducing extracellular Na (see Figure 10).

Ca²⁺ fluxes in cortical cells

Our suspensions contained 30–40% cells that did not stain with neutral red. These non-staining cells are thought to be non-chromaffin cells (Livett, 1985)

Figure 9 The dose-response curve for the action of pentobarbitone on the uptake of ^{22}Na stimulated by 500 μM carbachol. The points are the mean of 2–5 determinations and the vertical lines represent s.d.

Figure 10 The relationship between catecholamine (CA) secretion and ^{22}Na uptake during stimulation by 500 μM carbachol (CCh). (●) Show the relationship when secretion was inhibited by varying external Na^+ and (○) show the relationship in cell suspensions treated with varying concentrations of pentobarbitone (5–500 μM). The data are from three experiments.

which may be derived from islands of cortical cells present in the adrenal medulla. To determine the extent to which this contamination affected our results and conclusions we measured ^{45}Ca influx in suspensions of cortical cells treated with 500 μM carbachol or 77 mM K^+ . Carbachol had no effect on Ca^{2+} uptake whereas depolarization with 77 mM K^+ increased Ca^{2+} uptake with a time course similar to that seen for stimulated chromaffin cells. The uptake of ^{45}Ca was, however, lower than that for medullary cells ($232 \pm 15 \mu\text{M}$ per 10^6 cells per 10 min compared to $462 \pm 37 \mu\text{M}$ per 10^6 cells per 10 min) and this uptake was inhibited by 10% by 200 μM pentobarbitone. The ^{45}Ca uptake of medullary cells was inhibited by about 45% at 200 μM pentobarbitone (see Figure 1).

Discussion

The principal object of our experiments was to determine the mechanisms by which pentobarbitone inhibits neurosecretion using the chromaffin cell as a model. We showed that this anaesthetic decreases the secretion induced by carbachol and elevated concentrations of K^+ . This inhibition occurred at concentrations of pentobarbitone within the range required for the induction and maintenance of general anaesthesia (50–300 μM ; see Richards, 1972) and, at these concentrations, pentobarbitone had no significant effect on the basal secretion of catecholamine.

As the choice of the chromaffin cell as a model for neurosecretion was based on the homology between the adrenal medulla and sympathetic ganglia, it was

important to compare the action of pentobarbitone on the chromaffin cell with its action on synaptic transmission in sympathetic ganglia. The early work of Larrabee & Posternak (1952) showed that this anaesthetic, amongst others, selectively depressed synaptic transmission through the superior cervical ganglion. This effect occurred at concentrations of pentobarbitone comparable to those required for the maintenance of general anaesthesia. Later Matthews & Quilliam (1964) showed that the closely related barbiturate, amylobarbitone, depressed the release of acetylcholine in response to nerve stimulation. Barbiturates also depress the sensitivity of sympathetic ganglion cells to exogenously applied nicotinic agonists (Nicoll & Iwamoto, 1976). So in these important respects, the action of pentobarbitone on chromaffin cells parallels its action on sympathetic ganglia.

Depolarization of the cells with elevated concentrations of K^+ bypasses the acetylcholine receptor and directly activates the voltage-dependent Ca^{2+} channel. Under these conditions the influence of pentobarbitone on the process of transmitter secretion can be studied in isolation. The inhibition of catecholamine secretion induced by high- K^+ was accompanied by a decrease in Ca^{2+} influx. Moreover, the relationship between catecholamine secretion and Ca^{2+} influx during inhibition by pentobarbitone was the same as that observed between catecholamine secretion and Ca^{2+} influx determined by varying external Ca^{2+} . As pentobarbitone had no effect on the Ca^{2+} -activated secretion of catecholamine in electroporated cells (see Figure 8), we concluded that it does not affect those steps in exocytosis that occur after Ca^{2+} entry. The inhibition of Ca^{2+} entry by pentobarbitone appears to be sufficient to account in full for the inhibition of catecholamine secretion induced by direct depolarization and activation of the voltage-gated Ca^{2+} channel.

Our cell suspensions appeared to contain up to 40% non-chromaffin cells. These cells were probably adrenal cortical cells which showed a smaller increase in Ca^{2+} entry when they were depolarized by 77 mM K^+ than chromaffin cells. This contamination would, therefore, result in an underestimate of the Ca^{2+} entry for a given level of catecholamine secretion. Moreover, pentobarbitone was less effective in inhibiting Ca^{2+} entry in adrenal cortical cells. These two factors would result in an underestimate of the IC_{50} for the action of pentobarbitone on Ca^{2+} entry. Nevertheless, as our determination of the relationship between Ca^{2+} entry and catecholamine secretion was made under the same conditions, the conclusion that pentobarbitone inhibits catecholamine secretion by decreasing Ca^{2+} entry through the voltage gated channels remains valid.

Secretion of catecholamine from isolated chromaffin cells stimulated by carbachol was more sensitive to

the action of pentobarbitone than that stimulated by high K^+ . This confirms the observations of Holmes & Schneider (1973) on the effect of pentobarbitone on catecholamine secretion from intact glands. This difference in sensitivity indicated that, in addition to the effects occurring at the voltage-gated Ca^{2+} channel, there was a direct action of anaesthetic on the nicotinic receptor or on the processes associated with its activation.

We showed that pentobarbitone inhibited catecholamine secretion induced by acetylcholine and carbachol in a non-competitive manner and that it inhibited both ^{45}Ca and ^{22}Na influx. While the inhibition of Na^+ movement does not appear to be sufficient to account for the decrease in catecholamine secretion, the inhibition of ^{45}Ca influx paralleled the inhibition of catecholamine secretion. This suggests that pentobarbitone may inhibit Ca movements gated by the acetylcholine receptor as well as those of the voltage gated channel. However, the detailed mechanisms responsible for this effect remain to be worked out. It is known that pentobarbitone decreases the binding of acetylcholine to the acetylcholine receptor of the *Torpedo* electroplax (Sauter *et al.*, 1980) and this may also be true of its action on the nicotinic receptor of the chromaffin cell. However, it may act directly on the Na^+ channel associated with the nicotinic receptor as Adams (1976) has shown that, at the neuromuscular junction, barbiturates preferentially block ion channels in the open conformation possibly by rendering the open state less stable (Gage & McKinnon, 1985). The resolution of this issue in our preparation will depend on a detailed analysis of channel properties and receptor binding.

Although bovine chromaffin cells possess both nicotinic and muscarinic cholinergic receptors catecholamine secretion can only be induced by nicotinic agonists (Schneider *et al.*, 1977; Derome *et al.*, 1981). The muscarinic receptors may, however, modulate catecholamine secretion induced by nicotinic agonists (Derome *et al.*, 1981). As we used carbachol as our nicotinic agonist and as it possesses muscarinic properties it is possible that the decrease in catecholamine secretion caused by pentobarbitone may have resulted from modulation of cyclic GMP levels. This possibility was discounted as atropine (10^{-7} – 10^{-9} M) does not affect the potency of pentobarbitone (Pocock & Richards, unpublished observations). The possibility that the action of pentobarbitone might be mediated via activation of γ -aminobutyric acid (GABA) receptors (Kataoka *et al.*, 1984) was likewise rejected as catecholamine secretion is the same in the presence and absence of GABA (5–100 μ M) and the potency of pentobarbitone is the same in the absence and presence of GABA (Pocock & Richards, unpublished observations).

Implications for anaesthetic action in the CNS

In the CNS the nerve impulse depolarizes the nerve terminal and this opens voltage-sensitive Ca^{2+} channels to bring about a rise in free Ca^{2+} within the terminal which initiates transmitter release (Ashley *et al.*, 1984; Richards *et al.*, 1984). Electrophysiological analysis has demonstrated that concentrations of barbiturates within the anaesthetic range do not interfere significantly with the propagation of the nerve impulse, but they inhibit the release of putative neurotransmitters such as glutamate from synaptosomes and slices of brain tissue (see Collins, 1981; Minchin, 1981; Kendall & Minchin, 1982). Moreover, Blaustein & Ector (1974) have shown that concentrations of barbiturates within the anaesthetic range depress the K^{+} -stimulated Ca^{2+} influx into synaptosomes, but these authors did not correlate this decrease in Ca^{2+} flux with changes in transmitter release. In the present study we showed that the inhibition of Ca^{2+} influx was sufficient to account for the decrease in catecholamine secretion. As the mechanism of catecholamine secretion from chromaffin cells is thought to be similar to that of transmitter release from the nerve terminals of the CNS (see above), it is probable that the inhibition of Ca^{2+} influx is sufficient to account for the inhibitory action of

pentobarbitone on transmitter release in the CNS.

Pentobarbitone has been shown to affect the function of various receptors in the CNS. In the olfactory cortex, it depresses the sensitivity of pyramidal cells to iontophoretically-applied L-glutamate (Richards & Smaje, 1976), a putative transmitter in this pathway. It also depresses the sensitivity of hippocampal cells to glutamate (Sawada & Yamamoto, 1985). These results with glutamate are consistent with the known depressant effect of pentobarbitone on excitatory synaptic transmission in the hippocampus and olfactory cortex (Bliss & Richards, 1971; Richards, 1972).

In conclusion, we have shown that pentobarbitone inhibits the catecholamine secretion from bovine isolated adrenal chromaffin cells evoked by carbachol or by high K^{+} . This results from non-competitive inhibition of the nicotinic receptor and from inhibition of the voltage-gated Ca^{2+} entry. The decrease in Ca^{2+} influx accounts fully for the decrease in secretion in response to depolarization. A similar inhibition of the voltage sensitive Ca^{2+} channels could account for the depressant action of pentobarbitone on synaptic transmitter release in the CNS.

We wish to thank Ruth Shepherd for technical help and the Wellcome Trust for financial support.

References

- ADAMS, P. (1976). Drug blockade of open endplate channels. *J. Physiol.*, **260**, 531–552.
- ASHLEY, R.H., BRAMMER, M.J. & MARCHBANKS, R. (1984). Measurement of intrasynaptosomal free calcium by using the fluorescent indicator quin-2. *Biochem. J.*, **219**, 149–158.
- BAKER, P.F. & KNIGHT, D.E. (1981). Calcium control of exocytosis and endocytosis in bovine adrenal medullary cells. *Phil. Trans. R. Soc. Lond. Ser. B*, **296**, 83–103.
- BAKER, P.F. & KNIGHT, D.E. (1984). Calcium control of exocytosis in bovine adrenal medullary cells. *Trends in Neurosci.* **7**, 120–126.
- BLAUSTEIN, M.P. & ECTOR, A.C. (1975). Barbiturate inhibition of calcium uptake by depolarised nerve terminals *in vitro*. *Molec. Pharmacol.*, **11**, 369–378.
- BLISS, T.V.P. & RICHARDS, C.D. (1971). Some experiments with *in vitro* hippocampal slices. *J. Physiol.*, **214**, 7–9P.
- COLLINS, G.G.S. (1981). Release of endogenous amino acid neurotransmitter candidates from rat olfactory cortex: possible regulatory mechanisms and the effects of pentobarbitone. *Brain Res.*, **190**, 517–528.
- DEROME, G., TSENG, R., MERCIER, P., LEMAIRE, I. & LEMAIRE, S. (1981). Possible muscarinic regulation of catecholamine secretion mediated by cyclic GMP in isolated bovine adrenal chromaffin cells. *Biochem. Pharmacol.*, **30**, 855–860.
- GAGE, P.W. & MCKINNON, D. (1985). Effects of pentobarbitone on acetylcholine-activated channels in mammalian muscle. *Br. J. Pharmacol.*, **85**, 229–235.
- HOLMES, J.C. & SCHNEIDER, F.H. (1973). Pentobarbitone inhibition of catecholamine secretion. *Br. J. Pharmacol.*, **49**, 517–528.
- HOLZ, R.W., SENTER, R.A. & FRYE, R.A. (1982). Relationships between Ca^{2+} uptake and catecholamine secretion in primary dissociated cultures of adrenal medulla. *J. Neurochem.*, **39**, 635–646.
- KATAOKA, Y., GUTMAN, Y., GUIDOTTI, A., PANULA, P., WROBLEWSKI, J., COSENZA-MURPHY, D., WU, J.Y. & COSTA, E. (1984). Intrinsic GABAergic system of adrenal chromaffin cells. *Proc. natn. Acad. Sci. U.S.A.*, **81**, 3218–3222.
- KENDALL, T.J.G. & MINCHIN, M.C.W. (1982). The effects of anaesthetics on the uptake and release of amino acid neurotransmitters in thalamic slices. *Br. J. Pharmacol.*, **75**, 219–227.
- KNIGHT, D.E. & BAKER, P.F. (1982). Calcium-dependence of catecholamine release from bovine adrenal medullary cells after exposure to intense electric fields. *J. membr. Biol.*, **68**, 107–140.
- KNIGHT, D.E. & KESTVEN, N.T. (1983). Evoked transient intracellular Ca^{++} changes and secretion in isolated bovine adrenal medullary cells. *Proc. R. Soc. Lond. B.*, **218**, 177–199.
- LARRABEE, M.G. & POSTERNAK, J.M. (1952). Selective action of anaesthetics on synapses and axons in mammalian sympathetic ganglia. *J. Neurophysiol.*, **15**, 91–114.
- LIVETT, B. (1985). Adrenal medullary chromaffin cells *in vitro*. *Physiol. Rev.*, **64**, 1101–1161.

- MATTHEWS, E.K. & QUILLIAM, J.P. (1964). Effects of central depressant drugs upon acetylcholine release. *Br. J. Pharmac. Chemother.*, **22**, 415–440.
- MINCHIN, M.C.W. (1981). The effects of anaesthetics on the uptake and release of γ -amino butyric acid and D-aspartate in rat brain slices. *Br. J. Pharmac.*, **73**, 681–689.
- NICOLL, R.A. & IWAMOTO, E.T. (1978). Action of pentobarbital on sympathetic ganglion cells. *J. Neurophysiol.*, **41**, 977–986.
- POCOCK, G. (1980). Catecholamine secretion and ion movements in bovine adrenal medulla, Ph.D. Thesis, University of London.
- POCOCK, G. (1983a). Ionic and metabolic requirements for stimulation of secretion by ouabain in bovine adrenal medullary cells. *Molec. Pharmacol.*, **23**, 671–680.
- POCOCK, G. (1983b). Ion movements in isolated bovine adrenal medullary cells treated with ouabain. *Molec. Pharmacol.*, **23**, 681–697.
- POTASHNER, S., LAKE, N., LANGLOIS, E.A., PLOFFE, L. & LECAVALIER, D. (1980). Pentobarbital: differential effects on amino acid transmitter release. In *Molecular Mechanisms of Anesthesia*. Ed. B.R. Fink, pp. 469–476. New York, Raven Press.
- RICHARDS, C.D. (1972). On the mechanism of barbiturate anaesthesia. *J. Physiol.*, **227**, 749–767.
- RICHARDS, C.D. (1982). The actions of pentobarbitone, procaine and tetrodotoxin on synaptic transmission in the olfactory cortex of the guinea-pig. *Br. J. Pharmac.*, **75**, 639–646.
- RICHARDS, C.D., METCALFE, J.C., SMITH, G.A. & HESKETH, T.R. (1984). Free calcium levels and pH in synaptosomes during transmitter release. *Biochim. biophys. Acta*, **803**, 215–220.
- RICHARDS, C.D. & SMAJE, J.C. (1976). Anaesthetics depress the sensitivity of cortical neurones to L-glutamate. *Br. J. Pharmac.*, **58**, 347–357.
- RICHARDS, C.D. & STRUPINSKI, K. (1986). An analysis of the action of pentobarbitone on the excitatory postsynaptic potentials and membrane properties of neurones in the guinea-pig olfactory cortex. *Br. J. Pharmac.*, **89**, 321–325.
- SAUTER, J.F., BRASWELL, L.M. & MILLER, K.W. (1980). Action of anesthetics and high pressure on cholinergic membranes. In *Molecular Mechanisms of Anesthesia*. ed. Fink, B.R. pp. 199–207 New York: Raven Press.
- SAWADA, S. & YAMAMOTO, C. (1985). Blocking action of pentobarbital on receptors for excitatory amino acids in the guinea-pig hippocampus. *Exp. Brain Res.*, **59**, 226–231.
- SCHNEIDER, A.S., HERZ, R. & ROSENHECK, K. (1977). Stimulus-secretion coupling in chromaffin cells isolated from bovine adrenal medulla. *Proc. natn. Acad. Sci U.S.A.*, **74**, 5036–5040.
- VON EULER, U.S. & FLODING, T. (1955). A fluorimetric micromethod for differential estimation of adrenaline & noradrenaline. *Acta physiol. scand.*, Suppl 118, **33**, 45–56.
- WEAKLY, J.N. (1969). Effect of barbiturates on quantal synaptic transmission in spinal motoneurones. *J. Physiol.*, **204**, 63–77.
- WERZ, M.A. & MACDONALD, R.L. (1985). Barbiturates decrease voltage-dependent calcium conductance of mouse neurones in dissociated cell culture. *Molec. Pharmac.*, **28**, 269–277.

(Received April 25, 1986.

Revised September 13, 1986.

Accepted September 17, 1986.)