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The action of volatile anaesthetics on stimulus-secretion coupling in bovine adrenal chromaffin cells

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1 The action of four volatile anaesthetics, ethrane, halothane, isoflurane and methoxyflurane on stimulus-secretion coupling has been studied in isolated bovine adrenal medullary cells. All four agents inhibited the secretion of adrenaline and noradrenaline evoked by 500 μM carbachol at concentrations within the anaesthetic range. Total catecholamine secretion induced by stimulation with 77 mM potassium was also inhibited but at higher concentrations. All four agents inhibited the ^{45}Ca influx evoked by stimulation with 500 μM carbachol and the ^{45}Ca influx in response to K^+-depolarization.

2 When total catecholamine secretion in response to potassium or carbachol was modulated by varying extracellular calcium or by adding halothane or methoxyflurane to the incubation medium, the amount of catecholamine secretion for a given Ca^{2+} entry was the same.

3 The action of methoxyflurane on the relationship between intracellular free Ca and exocytosis was examined using electropermeabilised cells, which were suspended in solutions containing a range of concentrations of ionised calcium between 10^{-8} and 10^{-4} M. The anaesthetic had no effect on the activation of exocytosis by intracellular free calcium.

4 Halothane and methoxyflurane inhibited the carbachol-induced secretion of catecholamines in a non-competitive manner.

5 Halothane and methoxyflurane inhibited the increase in ^{22}Na influx evoked by carbachol. For halothane and methoxyflurane this inhibition of Na influx appears to be sufficient to account for the inhibition of the evoked catecholamine secretion.

6 We conclude that the volatile anaesthetics ethrane, halothane, isoflurane and methoxyflurane inhibit the secretion of adrenaline and noradrenaline induced by carbachol at concentrations that lie within the range encountered during general anaesthesia. In addition all four also inhibit the secretion of catecholamines induced by depolarization with 77 mM K^+ but at much higher concentrations. The decrease in Ca influx caused by methoxyflurane accounts fully for the decrease in secretion in response to depolarization with potassium. Similar actions at synapses within the CNS may underlie the general anaesthetic effects of these agents.

Introduction

Volatile anaesthetics such as halothane and methoxyflurane 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 chemical transmission is affected at low anaesthetic concentrations, rather than impulse conduction in the afferent fibres or the electrical properties of the postsynaptic cells (Richards, 1972; Richards *et al.*, 1975; Richards & White, 1975; Zorychta *et al.*, 1975). This implies that a significant part of their action involves a disturbance of neurosecretion, a reduction in the sensitivity of the postsynaptic receptors, or both.

Zorychta *et al.* (1975) showed that halothane decreases the quantal content of excitatory postsynaptic potentials (e.p.s.ps) recorded from spinal motoneurones, but there has been no detailed examination of the effect of volatile anaesthetics on the secretion of putative neurotransmitters. Within the CNS it has proved difficult to assign particular transmitters to specific pathways which can be studied readily *in vitro*. Clarification of this kind is,

however, an essential prerequisite for any detailed analysis of the action of anaesthetics on the mechanisms of transmitter release. To overcome this difficulty we have used adrenal chromaffin cells to examine the effects of four volatile anaesthetics on the secretion of catecholamines.

Chromaffin cells are derived from embryonic neural crest tissue and are homologous with sympathetic postganglionic neurones. They may be isolated in large numbers from bovine adrenal glands and form a relatively homogeneous population secreting adrenaline and noradrenaline in response to stimulation. In addition, it is possible to manipulate their intracellular environment using the technique of electropermeabilisation (Knight & Baker, 1982). The events leading to the secretion of catecholamines from chromaffin cells are well characterized (Baker & Knight, 1984). 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_i^{2+} which triggers granule exocytosis (Knight & Kesteven, 1983). Exposure of cells to depolarizing concentrations of K^+ bypasses the acetylcholine receptor and elicits secretion by opening the voltage-sensitive Ca^{2+} channels directly (Baker & Knight, 1981). Moreover, volatile anaesthetics are known to inhibit catecholamine secretion from adrenal glands both *in vivo* and *in vitro* (Gothert *et al.*, 1976; Gothert & Wendt, 1976). For these reasons, chromaffin cells offer a suitable experimental model for the analysis of the action of anaesthetics on neurosecretion.

We have made a detailed examination of the action of halothane and methoxyflurane on secretion induced by both pathways and of the effect of methoxyflurane on the relationship between intracellular free Ca^{2+} and exocytosis. We conclude that these agents depress the secretion induced by nicotinic stimulation by inhibiting the Na^+ influx through the channels gated by the nicotinic receptor and subsequent movement of Ca^{2+} through the voltage gated channel. All four agents inhibit Ca^{2+} influx in response to depolarizing concentrations of K^+ and this appears to be sufficient to account for the inhibition of secretion. A preliminary account of this work has been presented to the Physiological Society (Pocock & Richards, 1987b).

Methods

Isolation of chromaffin cells

Bovine adrenal glands were obtained from a local abattoir. They were removed from the animals within 40 min of death and perfused via the adrenal vein with 5–10 ml of ice-cold Locke solution containing 0.2% (w/v) bovine serum albumin. The glands were then placed on ice and transported to the laboratory.

Chromaffin cells were isolated by enzymatic digestion of thin slices of the adrenal medulla as described previously (Pocock, 1983). 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 rasies the possibility that Ca^{2+} fluxes induced by depolarization with high K^+ (but not by carbachol) might be due, in part, to influx into cortical cells (see Pocock & Richards, 1987a).

Measurement of catecholamine secretion and ion fluxes

Basal and evoked secretion of total catecholamines and the associated ion fluxes were measured by centrifuging cell suspensions through a layer of oil (see below). Total catecholamine (i.e. adrenaline plus noradrenaline) content was assayed by the method of Von Euler & Floding (1955) using a Perkin-Elmer fluorescence spectrophotometer.

All four anaesthetic agents are volatile so it was necessary to develop a protocol which minimized their loss from the incubation medium. Stock solutions of anaesthetic (10 mM in normal Locke or K^+-Locke) were prepared from liquid anaesthetic purified by distillation. The aqueous stocks were kept in tightly stoppered glass tubes with the minimum volume of air remaining above the solution. Appropriate dilutions were then made for individual experiments and these were also kept in tightly-stoppered glass tubes. A few minutes before the start of an experiment a small polycarbonate tube was 3/4-filled with normal Locke solution containing anaesthetic in an appropriate concentration and a dense suspension of cells added. The tube was then stoppered. Isotope ($^{22}Na^+$ or $^{45}Ca^{2+}$) was added to these cell suspensions which 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^+. The cell suspensions were incubated at 37°C with various concentrations of the anaesthetic for 5–10 min after which 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 total catecholamine content of the supernatant was assayed and expressed as a percentage of that present in the cell suspension after the cells had been lysed with 0.1% Triton X-100. Any fresh synthesis of adrenaline or noradrenaline during the experiment would therefore be automatically included in the total. The amount of isotope present in the cells was determined as described previously (Pocock, 1983).

With the large number of samples processed during each experiment it was not practical to estimate the anaesthetic content of each tube. Instead, we tested for the loss of anaesthetic during our procedure using independent controls. Samples of Locke solution which had been subjected to the procedure outlined above were passed over a C_{18} reverse phase liquid chromatography column. The amount of anaesthetic in the sample was measured by ultra-violet absorption at 208 nm. These experiments showed that our experimental procedure kept the loss of anaesthetics within 10% of the nominal values.

Electropermeabilised cells

A suspension of cells was washed in Ca^{2+}-free Locke solution containing 0.4 mM EGTA (pCa < 7) 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^{2+} is determined by that of the extracellular medium. The cells were then added to media containing methoxyflurane (0–1600 μM) and Ca and EGTA in varying ratios to define free Ca^{2+} at the desired levels (pCa was between 4 and 8). The amount of catecholamine (adrenaline plus noradrenaline) released into the medium after 10 min was measured and expressed as a percentage of the total present in the sample.

Physiological media

Normal Locke solution (Na-Locke) (mM): NaCl 140, KCl 5, $MgCl_2$ 1.8, $CaCl_2$ 1.0, HEPES 15 and 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 and for Na-free Locke, Na was replaced by an equimolar amount of N-methyl glucamine.

The composition of the electropermeabilisation medium was (mM): potassium glutamate 140, sodium glutamate 5, Mg-ATP 5.0, magnesium acetate 1.8, PIPES 15, EGTA 0.4 and glucose 5.0; and the pH was 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 methoxyflurane on catecholamine secretion

The total catecholamine content of our cell suspensions varied from 42 to 102 nmol per 10^6 cells with a mean value of 72 nmol per 10^6 cells. In the absence of anaesthetic, the amount of catecholamine present in the supernatant was 3–5% of the total and it increased slowly with time (approximately 2–3% per hour). We chose to stimulate the cells with 500 μM carbachol or 77 mM K^+ in most experiments as these conditions gave maximal responses (see Pocock & Richards (1987a) for details). The quantity of catecholamines released by the cells in response to either method of stimulation was similar. Evoked secretion in the absence of anaesthetic was generally 4–8% of total which corresponds to 2–6 nmol of catecholamine secreted per 10^6 cells and a basal secretion of 2% h^{-1} corresponds to approximately 1.4 nmol per 10^6 cells h^{-1}. This compares favourably with previously published values for freshly isolated chromaffin cells (Pocock, 1980; Derome *et al.*, 1981).

Addition of methoxyflurane (50–3200 μM) to the cell suspensions had no significant effect on basal catecholamine secretion, although the highest concentrations tested showed a slight rise compared to control. Methoxyflurane depressed the secretion evoked by 500 μM carbachol with an IC_{50} of 230 μM. It also inhibited the secretion evoked by 77 mM K^+ (IC_{50} around 1200 μM) (see Figure 1).

The action of methoxyflurane on ^{45}Ca influx

Freshly isolated bovine chromaffin cells have a resting influx of Ca^{2+} of about 15 μmol l^{-1}

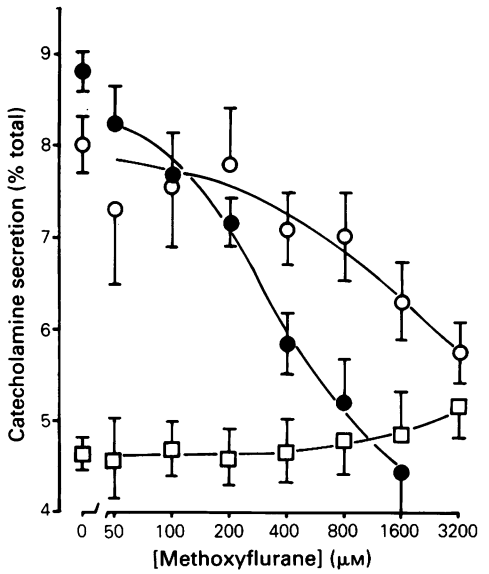

Figure 1 The dose-response relationship for the effect of methoxyflurane on basal catecholamine secretion (□) and on catecholamine secretion induced by 500 μM carbachol (●) and 77 mM potassium (○). The points shown are the means of 2–7 determinations and the vertical bars represent the s.e.

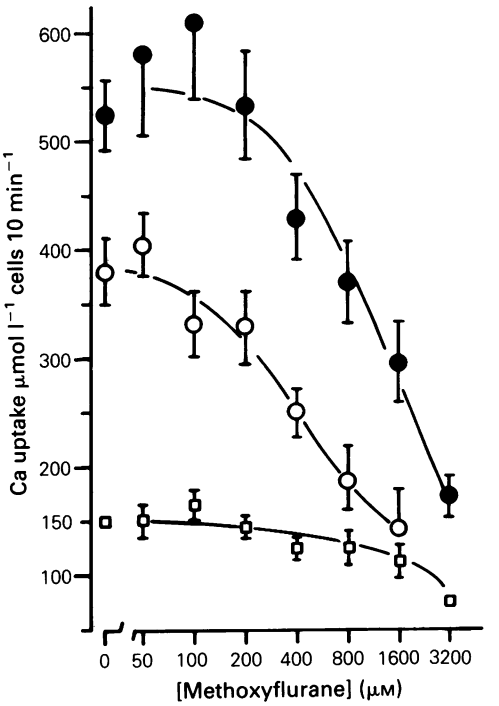

Figure 2 The dose-response relationship for the effect of methoxyflurane on basal ^{45}Ca influx (□) and on ^{45}Ca influx induced by 500 μM carbachol (○) and 77 mM potassium (●). The points shown are the means of 2–7 determinations and the vertical bars represent the s.e.

cells min^{-1}. When they are stimulated by carbachol or high K^+ the increase in secretion of catecholamine is associated with an increase in ^{45}Ca influx (Holz *et al.*, 1982). Methoxyflurane had little effect on basal Ca^{2+} uptake until its concentration exceeded 200 μM. Higher concentrations led to a progressive decrease in uptake (see Figure 2). The inhibition of evoked secretion by methoxyflurane was accompanied by a proportionate decrease in the ^{45}Ca influx evoked by carbachol or high K^+ (Figure 2).

Ca^{2+} influx and catecholamine secretion in response to stimulation are dependent on the extracellular Ca^{2+} concentration and it is thought that the Ca^{2+} influx acts as a trigger for catecholamine secretion. If methoxyflurane decreases catecholamine secretion primarily by inhibiting Ca^{2+} influx, we predict that the relationship between ^{45}Ca entry and the secretion of catecholamines in the presence of varying concentrations of anaesthetic at constant extracellular calcium (1 mM) should be the same as that determined in the absence of anaesthetic by varying extracellular calcium. For cells stimulated by depolarizing concentrations of K^+ the amount of catecholamine secretion for a given ^{45}Ca entry was similar under both sets of conditions. This suggests that methoxyflurane inhibits catecholamine secretion by inhibiting Ca^{2+} influx (see Figure 3).

The inhibitory effect of 250 μM methoxyflurane on both $^{45}Ca^{2+}$ influx and catecholamine secretion induced by nicotinic stimulation was apparent for all concentrations of carbachol tested (5–1000 μM). It was, therefore, non-competitive (not shown).

Methoxyflurane does not interfere with exocytosis

As methoxyflurane inhibits catecholamine secretion induced by high K^+ it could either be acting on the voltage-gated channel or on the intracellular events leading to exocytosis. To distinguish between these possibilities we have examined its action on the relationship between intracellular free Ca^{2+} and exocytosis in electropermeabilised cells. These cells were suspended in solutions containing a range of concentrations of Ca^{2+} between 10^{-8} and 10^{-4} M. The total amount of catecholamine secreted by the cells in the presence of methoxyflurane (300–1600 μM) was the same as that secreted by the cells in the absence of the anaesthetic. Methoxyflurane, therefore, had no effect on the intracellular events leading to exo-

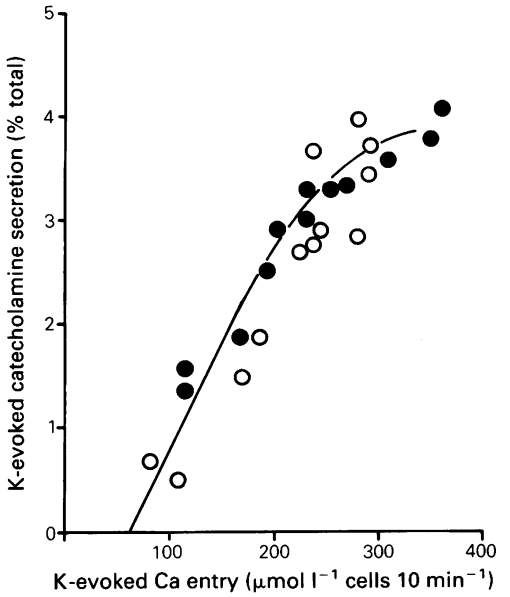

Figure 3 The relationship between catecholamine secretion and calcium uptake during stimulation by 77 mM potassium. (●) Show the relationship when external calcium is varied in the absence of methoxyflurane and (○) show the relationship in cell suspensions treated with varying concentrations of methoxyflurane. (The methoxyflurane concentrations used were: 100, 200, 400, 800 and 1600 μM and the calcium concentrations were 0.1, 0.25, 0.5, 1.0 and 2.0 mM). Data from a single experiment.

cytosis. The data for 1600 μM methoxyflorane are shown in Figure 4.

Effect of methoxyflurane on ^{22}Na influx

The total catecholamine secretion induced by carbachol was inhibited by 70% in the presence of 400 μM methoxyflurane. In contrast, the secretion induced by high K^+ was only partially inhibited even at 3200 μM methoxyflurane. These results suggest that methoxyflurane inhibits secretion by a direct effect on either the nicotinic receptor, the events associated with its activation or both. In an attempt to shed light on these effects we have investigated the action of methoxyflurane on the influx of ^{22}Na evoked by carbachol and correlated these measurements with the secretion of catecholamines.

The cells steadily accumulated ^{22}Na in the absence of stimulation (equivalent to 1.64 ± 0.43 mM Na l^{-1} cells min^{-1} (mean ± s.d., $n = 22$)). When the cells were stimulated by carbachol the influx of Na increased for 1–2 min. This evoked influx had a value of 7.88 ± 1.19 mM l^{-1} cells.

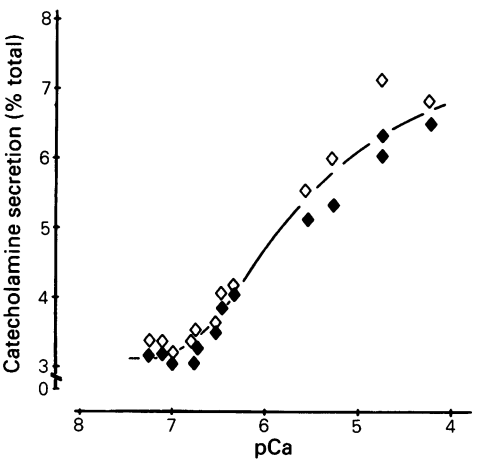

Figure 4 The influence of 1600 μM methoxyflurane on the activation of catecholamine secretion by ionised calcium in electropermeabilised chromaffin cells. At low external calcium (pCa < 6) the secretion was about 5% over a 15 min period. Raising ionised calcium levels from 10^{-7} to 10^{-4} (pCa 7–4) caused an increase in secretion. This pattern was not affected by methoxyflurane. (◆) Control responses; (◇) 1600 μM methoxyflurane.

Methoxyflurane did not affect the basal ^{22}Na influx but inhibited the increase in ^{22}Na influx elicited by carbachol in a dose-dependent way with an IC_{50} around 600 μM (see Figure 5).

To see whether this decrease in Na^+ influx was sufficient to account for the inhibition of secretion, we examined the relationship between ^{22}Na entry

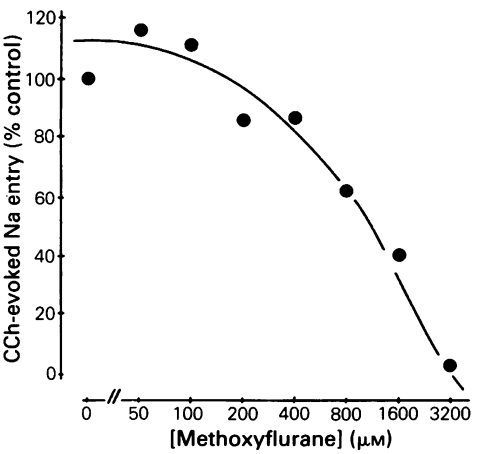

Figure 5 The dose-response curve for the action of methoxyflurane on the uptake of ^{22}Na stimulated by 500 μM carbachol (CCh). The data are from a single representative experiment.

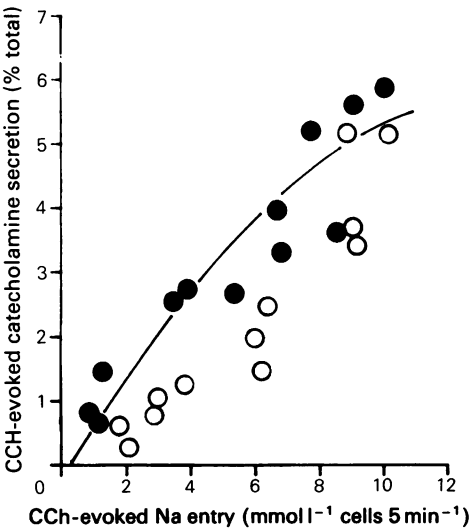

Figure 6 The relationship between catecholamine secretion and ^{22}Na uptake during stimulation by 500 μM carbachol (CCh). (●) Show the relationship when secretion was inhibited by varying external Na^+ and (○) show the relationship in cell suspensions treated with varying concentrations of methoxyflurane (100–1600 μM). The data are from a single experiment.

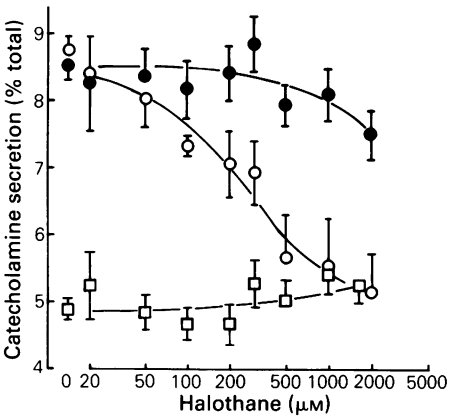

Figure 7 The dose-response relationship for the effect of halothane on basal catecholamine secretion (□) and on catecholamine secretion induced by 500 μM carbachol (○) and 77 mM potassium (●). The points shown are the means of 2–7 determinations and the vertical bars represent the s.e.

and total catecholamine secretion evoked by carbachol under the following conditions: (1) In the presence of varying concentrations of methoxyflurane with 147 mM sodium in the extracellular medium. (2) In the absence of anaesthetic with progressive replacement of extracellular sodium by the non-permeant cation N-methyl glucamine (meglumine).

During stimulation with carbachol, the amount of catecholamines secreted following a given entry of ^{22}Na was similar whether secretion was inhibited by methoxyflurane or by reducing extracellular Na (see Figure 6).

The actions of ethrane, halothane and isoflurane on catecholamine secretion and ion fluxes

Addition of ethrane (100–3200 μM), halothane (20–2000 μM) or isoflurane (100–3200 μM) to the cell suspensions had no significant effect on basal catecholamine secretion. The increase in secretion evoked by 500 μM carbachol was, however, inhibited by all three agents. In each case the extent of the inhibition was related to the concentration applied. The IC_{50} values were about 400 μM for ethrane, 300 μM for halothane and 450 μM for isoflurane. The secretion evoked by 77 mM K^+ could be inhibited by ethrane, halothane and isoflurane but this only occurred at concentrations greater than 1000 μM (see Table 1). The dose-response curve for the action of halothane on total catecholamine secretion is shown in Figure 7.

In unstimulated cells halothane had a weak concentration-dependent depressant effect on basal ^{45}Ca influx at concentrations greater than 100 μM. At the highest concentrations tested (2000–5000 μM) the decrease was 20–30% (see Figure 8). Ethrane, isoflurane and halothane inhibited the uptake of ^{45}Ca stimulated by carbachol in a dose-dependent manner. A similar pattern was seen with the K^+-stimulated ^{45}Ca uptake but, as with the catecholamine secretion, it was less sensitive to their action (see Table 1 and Figure 8). Halothane (300 μM) lowered the slope of the dose-response curve for carbachol-induced catecholamine secretion and ^{45}Ca influx, and decreased the maximum values obtained. Its effect was, therefore, non-competitive (not shown).

The relationship between ^{22}Na influx and total catecholamine secretion evoked by carbachol was examined in the presence of various concentrations of halothane with 147 mM Na in the extracellular medium and in the absence of anaesthetic with a progressive replacement of extracellular sodium by N-methyl glucamine. In agreement with the results obtained for methoxyflurane but in contrast to those for pentobarbitone (Pocock & Richards, 1987a), the amount of catecholamine secreted for a given entry of ^{22}Na was similar when secretion was inhibited by halothane or by reducing extracellular Na.

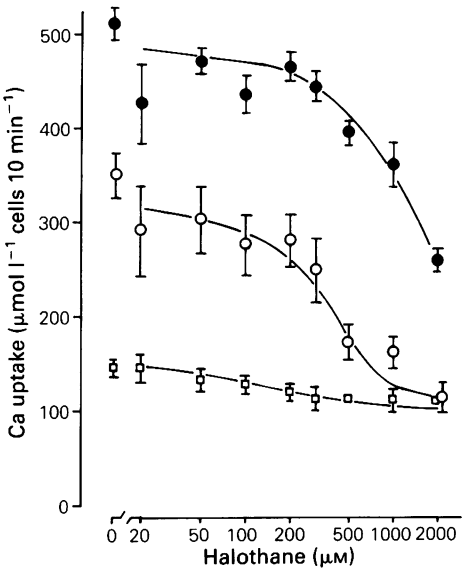

Figure 8 The dose-response relationship for the effect of halothane on basal ^{45}Ca influx (□) and on ^{45}Ca influx induced by 500 μM carbachol (○) and 77 mM potassium (●). The points shown are the means of 2–7 determinations and the vertical bars represent the s.e.

Discussion

The concentration of volatile anaesthetics in the extracellular fluid during general anaesthesia can be estimated from a knowledge of the amount of anaesthetic in the inspired air that is required to maintain a given depth of anaesthesia. A convenient standard is the minimum alveolar concentration (MAC) of an anaesthetic required to abolish motor activity in response to a strong noxious stimulus, such as a scalpel cut in the body wall. In man the MAC values for halothane and methoxyflurane are 0.75% and 0.16%, respectively. This concept assumes that a steady state has been established. The gas-water partition coefficient is 0.8 for halothane and 4.5 for methoxyflurane. From these data the concentrations of halothane and methoxyflurane in the extracellular fluid can be estimated as 285 μM and 320 μM, respectively. The equivalent data for ethrane and isoflurane are given in Table 1 (data from Steward *et al.*, 1973). Direct determinations of the arterial blood levels in dogs give values of 0.86–2.84 mM for halothane and 0.64–3.03 mM for methoxyflurane (Chenoweth *et al.*, 1962). After correction for the difference in partition coefficient between whole blood and water these values correspond to tissue fluid concentrations of 280–950 μM for halothane and 320–1240 μM for methoxyflurane. The MAC represents only one steady level of anaesthesia. A range of 0.5 × MAC to 2 × MAC is likely to cover most situations and this gives an anaesthetic range for all four agents of between 150–900 μM. The effects we have observed fall within these ranges (see Table 1).

The principal object of our experiments was to determine the mechanisms by which volatile anaesthetics interfere with stimulus-secretion coupling using the chomaffin cell as a model. The justification for this choice has been given in the Introduction and in a previous paper (Pocock & Richards, 1987a). We have shown that methoxyflurane and other volatile anaesthetics decrease the secretion induced by carbachol and that induced by depolarizing concentrations of K^+. The effects on the secretion evoked by carbachol occur at concentrations that lie within the range required for the induction and maintenance of general anaesthesia (see above) and, at these concentrations, none of the anaesthetics had a

Table 1 Concentrations of volatile anaesthetics required for the maintenance of general anaesthesia and for inhibition of catecholamine secretion in bovine adrenal medullary cells

	Ethrane	*Halothane*	*Isoflurane*	*Methoxyflurane*
MAC % inspired*	1.7%	0.75%	1.3%	0.16%
Equiv. aqueous concentration	540 μM	285 μM	330 μM	320 μM
Blood levels during anaesthesia**	—	0.86–2.84 mM	—	0.64–3.03 mM
IC_{50} for inhibition of CCh stimulation	400 μM	300 μM	450 μM	250 μM
IC_{50} for inhibition of high-K stimulation	3200 μM	>2000 μM	2200 μM	1200 μM

* Data from Steward *et al.* (1973).
** Data from Chenoweth *et al.* (1962).
MAC = minimum alveolar concentration and CCh = carbachol.

significant effect on the basal secretion of catecholamine.

Depolarization of the cells with elevated concentrations of K^+ bypasses the acetylcholine receptor and directly activates the voltage-dependent Ca^{2+} channels. Under these conditions the influence of anaesthetics on the process of catecholamine secretion can be studied in isolation. Although none of the anaesthetics used in this study have a strong depressant effect on catecholamine secretion induced by high-K^+, the decrease in evoked secretion is, in all cases, accompanied by a proportionate decrease in ^{45}Ca influx. Moreover, the relationship between total catecholamine secretion induced by depolarizing concentrations of K^+ and ^{45}Ca influx during inhibition by methoxyflurane is the same as that observed between total catecholamine secretion and ^{45}Ca influx determined by varying external Ca^{2+} (see Figure 3). As it appeared to have no effect on the Ca^{2+}-activated secretion of catecholamines in electropermeabilised cells (see Figure 4), we conclude that it does not affect those steps in exocytosis that occur after Ca^{2+} entry. So the inhibition of Ca^{2+} entry by methoxyflurane 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. A similar explanation would appear to suffice to account for the inhibition of K^+-evoked secretion caused by ethrane, halothane and isoflurane.

Secretion of catecholamines from isolated chromaffin cells stimulated by carbachol is more sensitive to the action of volatile anaesthetics than that stimulated by high-K^+. This confirms the observations of Gothert *et al.* (1976) and Gothert & Wendt (1976) on the effects of these agents on catecholamine secretion from intact glands. This difference in sensitivity is especially clear with halothane and indicates that, in addition to their effects on the voltage-gated Ca^{2+} channel, these anaesthetics have a direct action on the binding of nicotinic agonists to the receptor, on the processes associated with its activation or both.

We have shown that halothane and methoxyflurane depress catecholamine secretion induced by carbachol and that they inhibit both ^{45}Ca and ^{22}Na influx. The inhibition of Na^+ movement appears to be sufficient to account in full for the decrease in catecholamine secretion. One straightforward explanation for our findings is that the decrease in Na^+ entry gated by the receptor then leads to inhibition of the Ca entry through the voltage gated channel and so to inhibition of catecholamine secretion. It is also possible that the anaesthetics inhibit any Ca entry that occurs via the receptor-gated channel and that this in turn leads to inhibition of secretion. How these agents act on the receptor complex is unknown but we have also shown that halothane and methoxyflurane inhibit catecholamine secretion induced by carbachol in a non-competitive manner. The detailed mechanisms remain to be worked out. However, it is known that halothane increases the binding of acetylcholine to the acetylcholine receptor of the *Torpedo* electroplax (Firestone *et al.*, 1986) and this may also be true of its action on the nicotinic receptor of the chromaffin cell. If this is so, the higher affinity binding must reflect a desensitized state, as suggested by Firestone *et al.* (1986). Alternatively it, and the other agents we have studied, may act directly on the ion channel associated with the nicotinic receptor. The resolution of this issue will depend on a detailed analysis of channel properties and receptor binding.

Although bovine chromaffin cells possess both nicotinic and muscarinic cholinoceptors, catecholamine secretion can only be induced by nicotinic agonists (Schneider *et al.*, 1977; Derome *et al.*, 1981). The muscarinic receptors may modulate catecholamine secretion induced by nicotinic agonists (Derome *et al.*, 1981). However, this possibility was discounted as atropine (10^{-7}–10^{-9} M) did not affect the amount of catecholamine secreted in response to carbachol under the conditions employed in our experiments (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, in turn, initiates transmitter release (Ashley *et al.*, 1983; Richards *et al.*, 1984). Electrophysiological analysis has shown that concentrations of halothane and methoxyflurane within the anaesthetic range inhibit excitatory synaptic transmission (Richards, 1972; Richards *et al.*, 1975; Zorychta *et al.*, 1975). Here we have shown that these anaesthetics depress the secretion of catecholamines in response to carbachol at lower concentrations than those required to inhibit secretion induced by high K^+. This suggests that their site of action will be at the postsynaptic receptors rather than at the presynaptic nerve ending. This is consistent with the depressant effect of methoxyflurane on the sensitivity of olfactory neurones to the putative excitatory transmitter glutamate, but contrasts with the conclusion reached by Richards & Smaje (1976) and Zorychta *et al.* (1975) for the site of action of halothane; they suggested that it exerted its depressant effects on excitation in the CNS by a presynaptic action.

The inhibition of the voltage-gated Ca^{2+} influx seen with all four agents is sufficient to account for the decrease in catecholamine secretion induced by

depolarization. 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 would result in a decrease in the amount of transmitter released in response to a nerve volley. However, on the basis of this study, it appears that such actions would make only a small contribution to the blockade of excitatory synaptic transmission produced by general anaesthetics during the maintenance phase of anaesthesia, while the inhibition of receptor-mediated events would be the major effect.

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