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Interactions of cholinesterase inhibitors and glucocorticoids with ketamine and pentobarbitone-induced general anaesthesia in the rat: possible effects on central cholinergic activity

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1 Doses of 100, 150 and 200 $\mu g\,kg^{-1}$ of the cholinesterase inhibitor neostigmine reverse the anaesthetic action of ketamine. The antagonistic effect is increased as the dose is increased. The duration of anaesthesia induced by pentobarbitone is reversed by the cholinesterase inhibitor in doses of 150, 200 and 250 $\mu g\,kg^{-1}$.

2 Choline, in a dose of 50 $mg\,kg^{-1}$, significantly antagonizes the action of the two anaesthetics, whereas hemicholinium-3, an inhibitor of the uptake of choline and the synthesis of acetylcholine, markedly potentiates their action.

3 Dexamethasone induces a significant reduction of the duration of anaesthesia produced by ketamine and pentobarbitone. The potentiation of the anaesthetic effect caused by hemicholinium-3 is also reversed by dexamethasone.

4 The acetylcholine content in rat cerebral cortex is increased after treatment with ketamine and pentobarbitone.

5 Measurements of the course of the plasma level of pentobarbitone do not reveal alterations in the pharmacokinetic profile by either neostigmine or dexamethasone.

6 These results indicate that central cholinergic systems may somehow be involved in the anaesthesia induced by ketamine and pentobarbitone and that the interactions described in this paper may be the result of modification by neostigmine and dexamethasone of the alterations in cholinergic activity caused by the two anaesthetics.

Introduction

In 1977 Balmer & Wyte showed that physostigmine antagonized the sedative effects of diazepam and droperidol. In addition these authors found that patients receiving an infusion of ketamine, 50 $\mu g\,kg^{-1}\,min^{-1}$, came round immediately after an intravenous injection of 0.5 mg physostigmine. The analgesic state remained unaltered, but the unpleasant sensations, characteristic of ketamine-anaesthesia, were suppressed.

Subsequent to this paper, these observations were denied as well as confirmed by other authors. Drummond *et al.* (1979) concluded from the results of a double blind clinical trial in 110 patients, that physostigmine did not shorten the duration of anaesthesia caused by ketamine and did not suppress the after-effects induced by the anaesthetic. Recovery time after completion of the operation was actually prolonged. In contrast Toro-Matos *et al.* (1980) reported that physostigmine significantly reduced the duration of ketamine-induced anaesthesia in man.

Other authors investigated these clinically significant findings in animals. Livingstone & Waterman (1978) found that the duration of anaesthesia induced by ketamine in rats, is reduced by physostigmine, whereas it is prolonged by atropine. One dose of each drug was investigated. Foote & Livingstone (1978) observed that physostigmine shortens and atropine prolongs the duration of anaesthesia produced by both ketamine and pentobarbitone in male rats. Lawrence & Livingstone (1979) confirmed the findings that physostigmine, and also neostigmine, antagonize the actions of ketamine in rats. However,

Figallo & Wingard (1979) showed that in male rats the anaesthetic action of ketamine was significantly prolonged by physostigmine.

The purpose of the study to be presented in this paper was to investigate the above described interactions more closely and in a systematic way. In addition preliminary attempts were made to elucidate the possible mechanism(s) underlying these interactions.

Methods

Female outbred rats of the Cpb: Wu(Wi) strain, weighing 150–170 g, were used, all receiving food and water *ad libitum*. Unless otherwise stated each treatment group consisted of nine to twelve animals. All drugs were administered by intraperitoneal (i.p.) injection. When combinations of drugs were given, the drugs were administered in different syringes. Experiments measuring the duration of anaesthesia ('sleeping time') were performed in a special room with a constant temperature (23°C) and humidity (60%). The body temperature of the test animals was measured, and usually decreased with an average of 3°C. Duration of anaesthesia was defined as the lapse of time between the moment the righting reflex was lost and the moment the animal turned over spontaneously.

Acetylcholine content in rat cortex was measured as follows. Acetylcholine (total) was extracted according to a modification of the method described by Beani & Bianchi (1963). Rats were decapitated and the skull, placed on ice, was opened and 300–700 mg cortex tissue rapidly excised. The tissue was weighed and dropped in a Potter-Elvehjem homogenizer and 2 ml McIlvaine buffer (0.1 M citric acid + 0.2 M NaH_2PO_4, pH 4.0) added. The Potter homogenizer was placed in boiling water for 3 min in order to inactivate enzymes and other active substances (acetylcholine is highly stable at pH 3.8–4.5). After cooling the tissue was carefully homogenized and the pestle and wall of the tube washed with 2 ml McIlvaine buffer. The homogenate was left at room temperature for 15 min and subsequently centrifuged at 3000 r.p.m. The supernatant was transferred to a calibrated tube and the pellet washed with a same quantity of Krebs Ringer solution with a double concentration of salts, but without glucose and $NaHCO_3$ (in order to obtain an isotonic medium). The mixture was centrifuged at 3000 r.p.m. and the supernatant decanted in the calibrated tube. The volume was recorded and used for conversion. The extract was assayed on the guinea-pig ileum against a freshly prepared solution of acetylcholine.

The acetylcholine content in rat cortex was assayed, using the guinea-pig ileum (according to the four point assay with doses arranged according to a Latin square) in an organ bath filled with Krebs Ringer solution, areated with 95% O_2 + 5% CO_2 at 34°C. The Krebs Ringer solution contained (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, and glucose 11.1. Contractions were recorded with a displacement transducer for isotonic contractions (7 DCDM-1000 Hewlett and Packard, California, U.S.A.) In a number of preliminary experiments, with infrequent intervals, acetylcholine was assayed in the presence of mepyramine and methysergide, but this procedure had no influence on the sensitivity of the ileum or on the outcome of the assay, as compared to a simultaneous assay without addition of the antagonists.

Qualitative analysis (cascade of preparations) had previously indicated that acetylcholine was the only (known) agonist present in large quantities in the extract. Atropine (10 $\mu g\,ml^{-1}$) inhibited completely contractions of the ileum caused by the extract. Moreover, in a few experiments a biological assay of acetylcholine was carried out using the stomach fundus strip of the rat with methysergide present in the Krebs Ringer solution. Values of these assays were identical to those obtained from assays of the same extracts, using the guinea-pig ileum.

A number of rats were injected with pentobarbitone 60 $mg\,kg^{-1}$ ($n=3$), or pentobarbitone 60 $mg\,kg^{-1}$ simultaneously with neostigmine 200 $\mu g\,kg^{-1}$ ($n=3$), physostigmine 200 $\mu g\,kg^{-1}$ ($n=3$) or dexamethasone 500 $\mu g\,kg^{-1}$ ($n=3$). At fixed time intervals plasma samples were taken from a cannula inserted in the carotid artery (0.25 ml blood each time) for the determination of plasma concentrations of pentobarbitone. The blood pressure was measured, and it was noted that the loss of this amount of blood each time did not affect the pressure.

For determination of pentobarbitone the following extraction procedure was employed. To 50 μl plasma, 0.4 ml of a 10 $\mu g\,ml^{-1}$ hexobarbitone solution in 0.05 M sodium acetate, pH 5.5, was added as an internal standard. For extraction of pentobarbitone and hexobarbitone 2 ml of a mixture of *n*-hexane, diethylether and (−)-propanol (HEP), 49:49:2 v/v), was used (Tjaden *et al.*, 1977). Mixing was performed on a supermixer, 3 times for 5 s. After separation of the two phases on standing, the HEP-layer was removed and evaporated to dryness at room temperature with a stream of filtered air. The samples were dissolved in 200–400 μl methanol-water, 51:49 (v/v). High pressure liquid chromatography (h.p.l.c.) analysis was performed with a Perkin Elmer 3B chromatograph, LC-75 spectrophotometric detector at 217 nm and Sigma 10 B data station, using a Lichrosorb 10 RP 18 column (4.6 × 250 mm) from Chrompack. The column was eluted with a mixture of methanol and water, 51:49 (v/v),

$2\,ml^{-1}$ min, and 100 µl samples were injected. Each experiment was performed twice. Pentobarbitone was determined by peak height ratio analysis using a Hewlett-Packard 9815 A calculator programmed for linear regression and correlation. Calibration graphs were obtained by adding 1.5, 3.0, 6.0 and 9.0 µg of pentobarbitone to 50 µl of blank rat plasma. For every new series of experiments a new calibration graph was constructed. Correlation coefficients usually were at least 0.999.

Results were analyzed statistically using Student's *t* test.

Results

Duration of anaesthesia

The effect of neostigmine on the duration of anaesthesia in rats induced by ketamine and pentobarbitone can be seen in Figure 1. Doses of 100 and 150 $\mu g\,kg^{-1}$ neostigmine slightly, but significantly reduce the duration of anaesthesia caused by ketamine ($P<0.05$), whereas a dose of 200 $\mu g\,kg^{-1}$ is highly effective ($P<0.005$) especially in antagonizing the action of the highest doses of ketamine (Figure 1a).

All doses of neostigmine used (150, 200 and 250 $\mu g\,kg^{-1}$) are highly effective in antagonizing the anaesthetic action of pentobarbitone in rats (Figure 1b), at the highest doses (30 and 40 $\mu g\,kg^{-1}$) of the latter ($P<0.005$).

We next studied the effects on the duration of anaesthesia of some drugs known to affect the uptake of choline and its incorporation into acetylcholine into nerve endings (see Veldsema-Currie *et al.*, 1976; Leeuwin *et al.*, 1978), i.e. choline, hemicholinium-3 (HC-3) and the corticosteroid dexamethasone (see under Discussion).

As can be seen in Figure 2a, both choline (50 $mg\,kg^{-1}$) and dexamethasone (500 $\mu g\,kg^{-1}$) significantly antagonize the anaesthetic action of all doses of ketamine used ($P<0.005$), whereas HC-3 in a dose of 150 $\mu g\,kg^{-1}$ causes a marked prolongation of the sleeping time. This prolongation is some-

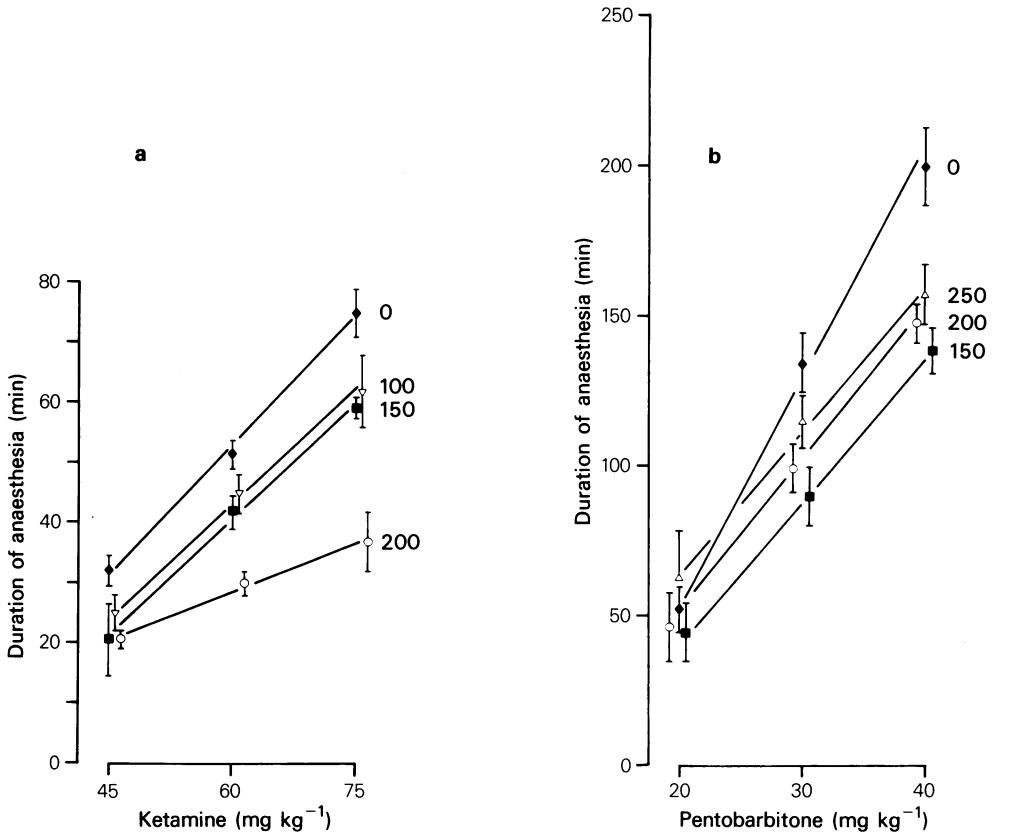

Figure 1 Effects of various doses of neostigmine (indicated in figure as $\mu g\,kg^{-1}$) on the dose-response curves of ketamine (a) and pentobarbitone (b) with regard to the duration of anaesthesia in rats. Values are mean with s.e.mean indicated by vertical lines.

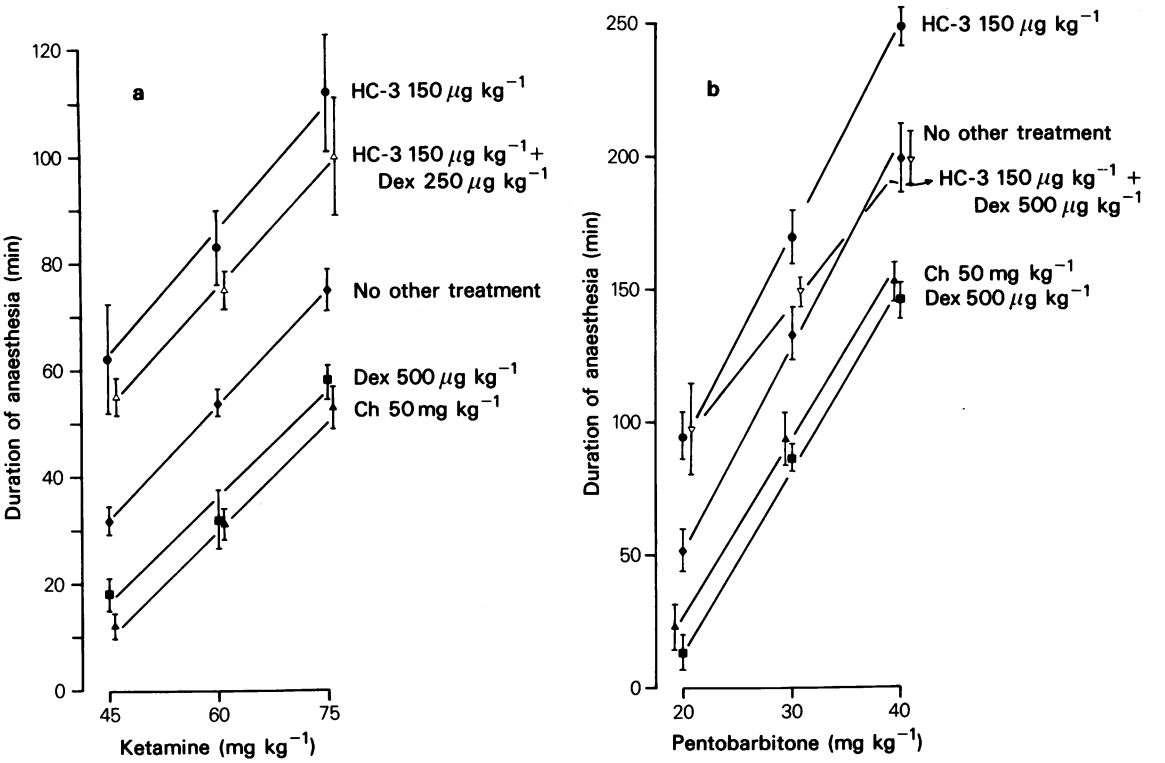

Figure 2 Effects of dexamethasone (Dex), choline (Ch), hemicholinium-3 (HC-3) and HC-3 + Dex on the dose-response curves of ketamine (a) and pentobarbitone (b) with regard to the duration of anaesthesia in rats; values are mean with s.e.mean indicated by vertical lines.

what reduced by dexamethasone, 250 $\mu g\,kg^{-1}$, although the difference is not statistically significant ($P > 0.05$).

Choline, 50 $mg\,kg^{-1}$, and dexamethasone, 500 $\mu g\,kg^{-1}$, also cause a reduction of the pentobarbitone-induced duration of anaesthesia, although this effect is less marked than in the case of ketamine (Figure 2b). HC-3, 150 $\mu g\,kg^{-1}$, significantly potentiates the anaesthetic action of pentobarbitone ($P < 0.005$), whereas 500 $\mu g\,kg^{-1}$ dexamethasone, at the highest dose of the anaesthetic agent, completely reverses the action of HC-3 ($P < 0.005$). A dose of 250 $\mu g\,kg^{-1}$ of the corticosteroid is ineffective and higher doses of dexamethasone, e.g. 1000 $\mu g\,kg^{-1}$, have an opposite effect; they tend to potentiate the action of HC-3.

Acetylcholine content of rat cortex

The effects of several doses of ketamine have been tested. The results are shown in Figure 3a. Between 100 and 200 $mg\,kg^{-1}$, ketamine produces a dose-related increase of the acetylcholine content of rat brain cortex 40 min after the loss of the righting reflex. This rise also appears to be time-related (Figure 3b): with an increase of the action of ketamine, 75 $mg\,kg^{-1}$, the acetylcholine content increases steadily. Lower doses than 75 $mg\,kg^{-1}$ were tested as well, and although they were effective in increasing the acetylcholine level, no clear-cut dose-effect relations could be established.

Figure 4 shows that treatment with pentobarbitone also causes a dose-related increase of the acetylcholine content of the cerebral cortex. At a dose of 20 $mg\,kg^{-1}$ pentobarbitone a number of animals either did not lose the righting reflex or came round before 40 min. All these animals had more or less 'normal' acetylcholine levels in their cerebral cortex, varying between 3.36–5.73 $\mu g\,g^{-1}$ brain cortex. These values were excluded from the calculation of the average.

The course of the pentobarbitone concentration in rat plasma

Figure 5 shows six separate curves indicating the course of the plasma concentrations of pentobarbitone, after treatment of animals with 60 $mg\,kg^{-1}$

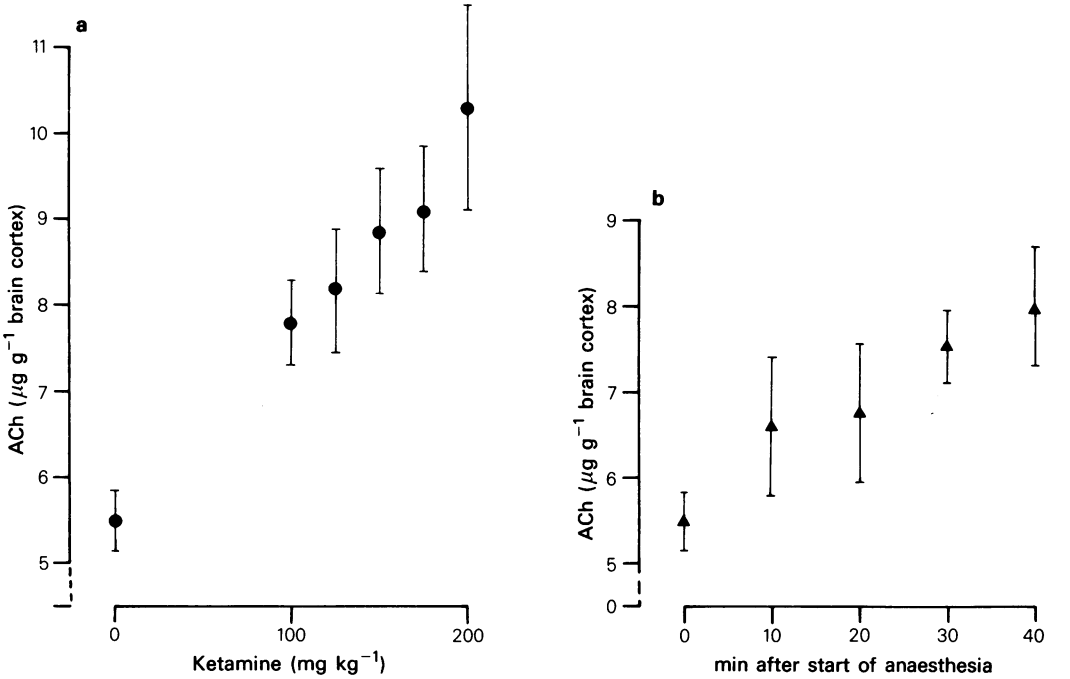

Figure 3 (a) Effects of various doses of ketamine on the acetylcholine (ACh) content of rat cerebral cortex. Animals were killed 40 min after start of anaesthesia. (b) Effects of ketamine 75 mg kg^{-1} on the acetylcholine content of rat cerebral cortex at various points in time during anaesthesia. All points are mean with s.e.mean indicated by vertical lines.

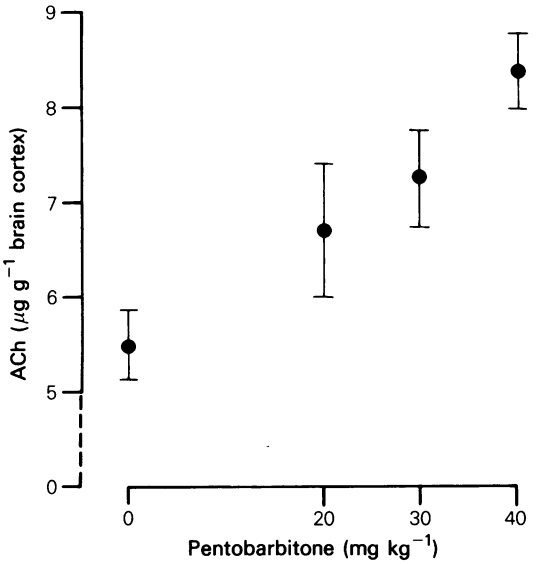

Figure 4 Effects of various doses of pentobarbitone on the acetylcholine (ACh) content of rat cerebral cortex. Animals were killed 40 min after start of anaesthesia; values are mean with s.e.mean indicated by vertical lines.

pentobarbitone alone (complete lines) or 60 mg kg^{-1} pentobarbitone simultaneously with 200 μg kg^{-1} neostigmine (broken lines). The blood pressure of the animals is not significantly affected by neostigmine. As can be seen, there are no marked differences between the two sets of curves, indicating that the cholinesterase inhibitor neostigmine in no way affects the kinetic profile of pentobarbitone in blood plasma.

Similar experiments were performed with physostigmine or dexamethasone instead of neostigmine. Neither physostigmine, nor dexamethasone induce any obvious change in the course of the plasma concentrations of pentobarbitone.

Discussion

The reason why neostigmine was used in this study calls for some comment. It can be argued that neostigmine as a quaternary ammonium compound would not be expected to cross the blood-brain barrier. However, physostigmine which is known to cross the blood-brain barrier easily, could not be used in our experiments, since it was noted in preliminary experiments, that animals which received physostigmine in

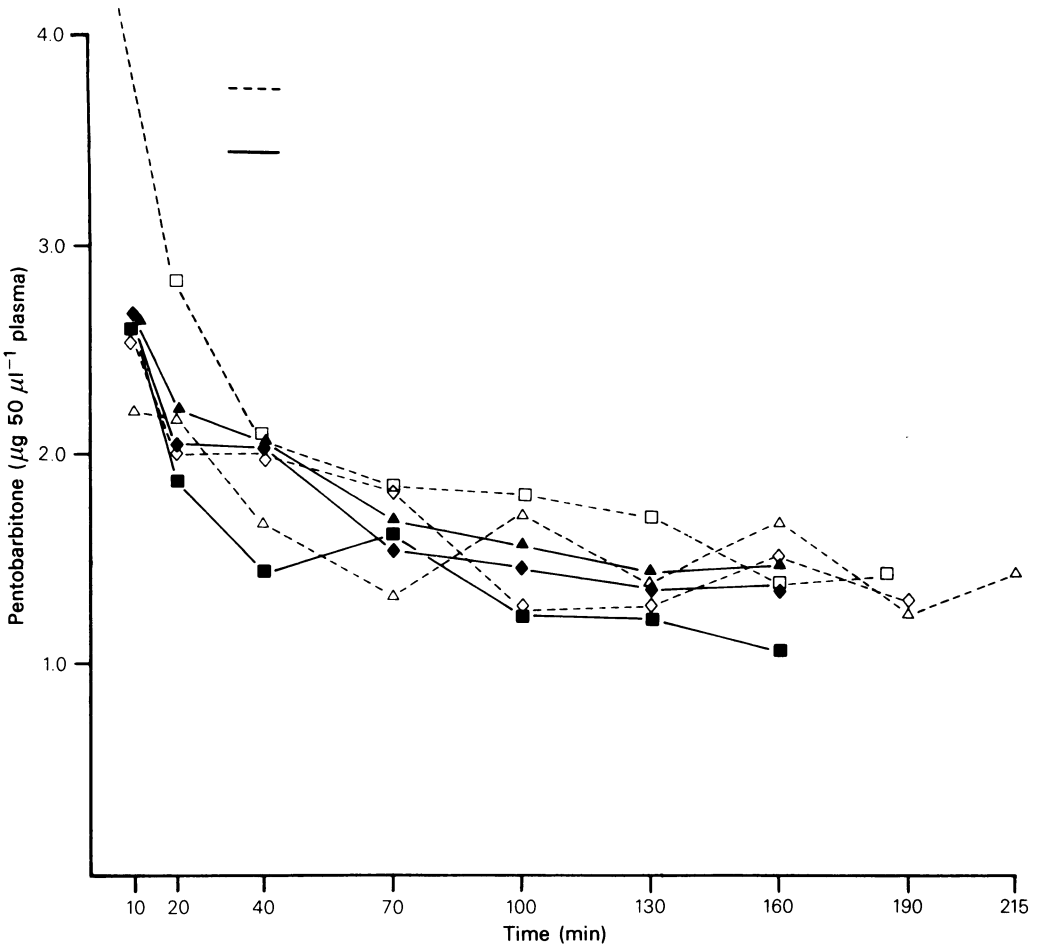

Figure 5 Effect of simultaneous administration of neostigmine (i.p.) on the course of the plasma concentration of pentobarbitone (i.p.). Each line represents one animal. Each point is the average of two determinations. Complete lines indicate pentobarbitone 60 mg kg^{-1} alone; broken lines indicate pentobarbitone 60 mg kg^{-1} plus neostigmine 200 μg kg^{-1}.

doses that displayed interactions with the anaesthetics, showed severe toxic effects due to cholinesterase inhibition. The doses of neostigmine used produced far less toxic signs than physostigmine. Another point of consideration was that Lawrence & Livingstone (1979) had previously shown that neostigmine, like physostigmine was effective in antagonizing anaesthetic effects of ketamine in rats; moreover, we have shown that the same is true for the anaesthetic effects of ketamine and pentobarbitone respectively, in mice (Leeuwin *et al.*, 1982). Furthermore Schweitzer & Wright showed, as early as 1938, that neostigmine in 'small' as well as in 'large' doses has respiratory effects in cats, due to central actions; Siskel Frey & Gesell (1948) reported that both physostigmine and neostigmine in 'moderate' doses produced hyperpnoea, which the authors attributed to central potentiation of nervous activity. Finally Bradley & Elkes (1953, 1957) have shown that when neostigmine is administered systemically to cats in certain doses (200–400 μg kg^{-1}), it may have effects on the electrical activity of the brain.

Animal studies of Livingstone & Waterman (1978), Foote & Livingstone (1978), Lawrence & Livingstone (1979), Avant *et al.* (1979) and Toro-Matos *et al.* (1980), supported and those of others (Drummond *et al.*, 1979; Figallo & Wingard, 1979) conflicted with the observations of Balmer & Wyte (1977) in man that the cholinesterase inhibitor, physostigmine, antagonizes the anaesthetic action of ketamine. Most of these studies were performed using only one dose of the anaesthetic and usually, also not more than a single dose of a cholinesterase inhibitor. In some studies no mention was made of

the sex of the animals used, which may be of importance, since sex differences in the metabolism of anaesthetics may exist.

The present study shows that interactions between cholinesterase inhibitors and general anaesthetics are dependent on the dose of both the anaesthetic drug, and the cholinesterase inhibitor.

Although the cholinesterase inhibitor, neostigmine, is capable of antagonizing the anaesthetic effects of ketamine and pentobarbitone as judged from the reduction of the duration of anaesthesia, there are some restrictions to be made, which may explain the conflicting data of others.

In the case of ketamine, antagonism of the anaesthetic action is directly proportional to the dose of the cholinesterase inhibitor used, but most striking at the highest dose levels of ketamine. Pentobarbitone-induced anaesthesia is only antagonized markedly (statistically highly significant) at its highest dose. A possible explanation for the phenomenon that a significant effect of the cholinesterase inhibitor becomes manifest only at the higher dose levels of the anaesthetics may be that at lower doses of the latter, i.e. $45\,mg\,kg^{-1}$ ketamine or $20\,mg\,kg^{-1}$ pentobarbitone, anaesthesia is not yet induced, only loss of righting reflex. Duration of loss of righting reflex alone is not affected by neostigmine, but when anaesthesia is produced by anaesthetic doses of ketamine or pentobarbitone, this latter effect is antagonized. These observations however, may explain the highly controversial findings of other authors.

These remarkable dose-effect relations of neostigmine may be compared with the effects observed by Veldsema-Currie *et al.* (1976) and Leeuwin *et al.* (1978). These authors showed that choline uptake and the incorporation of choline into acetylcholine in the endplate-rich area of rat diaphragm *in vitro* can be stimulated at relatively low doses and inhibited by higher doses of the cholinesterase inhibitors, neostigmine and physostigmine. Moreover, Atweh *et al.* (1975) found that both cholinesterase inhibitors decrease the choline uptake in the cerebral cortex *in vitro*.

Elliott *et al.* (1950) found the acetylcholine content in rat and cat brain tissue increased after *in vivo* treatment with pentobarbitone. Crossland & Merrick (1954) showed that the amount of extractable acetylcholine in the brain of rabbits, rats and mice increased as a result of anaesthesia with a number of anaesthetics. Richter & Walker (1977) observed an elevation of the level of acetylcholine in rat cerebral cortex after pentobarbitone. Simon *et al.* (1976) reported that pentobarbitone produces a reduction of the sodium-dependent high affinity choline uptake in synaptosomes from hypothalamus and cerebral cortex of the rat *in vitro*. According to these authors this high affinity accumulation rate is a measure for activity in cholinergic nerve endings. Using hippocampal synaptosomes Richter *et al.* (1982) found the same during anaesthesia with pentobarbitone.

It was shown by Veldsema-Currie *et al.* (1976) that HC-3 inhibits the uptake of radioactive choline and its incorporation into acetylcholine, while the corticosteroid, dexamethasone, at low doses reduces (but at higher doses increases) the inhibition by HC-3 of the uptake of choline and its incorporation into acetylcholine in the endplate-rich area of rat diaphragm. Comparable effects were shown in the present investigation. Choline as well as dexamethasone, caused a significant antagonism of the anaesthesia induced by ketamine and pentobarbitone, whereas HC-3 markedly prolonged the action of the two anaesthetics. Dexamethasone reversed this prolongation, but it is of interest to note that relatively high doses of the hormone intensified the effect of HC-3.

The present study has shown furthermore that ketamine produces a dose- and time-related elevation of the acetylcholine content of rat cerebral cortex. Treatment with different doses of pentobarbitone caused a similar increase of the acetylcholine content of the cortex. These results are in full agreement with early findings reported by Elliott *et al.* (1950), using pentobarbitone as an anaesthetic, and the results of Crossland & Merrick (1954) using other anaesthetics such as ether and chloralose. Elliott *et al.* (1950) explained the rise of the acetylcholine level, by assuming that influences which decrease the activity of cholinergic neurones, thus inhibiting the release, allow the reserve of bound acetylcholine to build up to abnormally high levels. Conversely influences which stimulate nervous activity might be expected to diminish the acetylcholine stores.

The fact that anaesthetics increase the acetylcholine content of rat cerebral cortex, combined with the results which showed that compounds such as choline, dexamethasone and cholinesterase inhibitors, which facilitate and compounds such as HC-3, dexamethasone (under certain conditions) and cholinesterase inhibitors (under certain conditions), which inhibit the choline uptake and the synthesis of acetylcholine, antagonize or potentiate the action of general anaesthetics, may indicate that cholinergic mechanisms are somehow involved in the anaesthesia caused by the anaesthetics used, and that cholinesterase inhibitors (and corticosteroids) may interact with these anaesthetics on the basis of alterations in activity of cholinergic neurones in the cerebral cortex.

At the moment we have no satisfactory explanation for the effect of neostigmine and for this kind of dose-relationship of neostigmine with the anaesthetic effects of ketamine or pentobarbitone. However,

work is now in progress, measuring direct effects of anaesthetics alone or in combination with cholinesterase inhibitors (or corticosteroids), in various dosages, on central cholinergic mechanisms. It is possible that these data may deepen our insight in the mechanisms underlying the antidotal actions of cholinesterase inhibitors.

In order to determine whether the interaction of neostigmine with the two general anaesthetics used might be of pharmacokinetic origin, plasma levels of pentobarbitone after i.p. injection in animals which had been simultaneously treated i.p. with neostigmine were determined. It was shown that the pharmacokinetic profile of pentobarbitone in blood was not altered by simultaneous treatment with neostigmine, nor was it altered by simultaneous treatment with physostigmine or dexamethasone.

These observations justify the assumption that cholinesterase inhibitors display no obvious pharmacokinetic interaction with general anaesthetics. It is thus reasonable to assume that, like physostigmine and dexamethasone, at least part of neostigmine must cross the blood-brain barrier to act at certain central nervous structures.

At the moment it is tentatively concluded that cholinergic systems may be involved in the anaesthesia caused by ketamine and pentobarbitone and that cholinesterase inhibitors and corticosteroids modify changes induced by the anaesthetics in these systems.

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