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EFFECTS OF ANESTHETICS ON SODIUM UPTAKE INTO RAT BRAIN CORTEX *IN VITRO*

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Abstract—Local anesthetics (cocaine, lidocaine) suppress the uptake of Na^+ into rat brain cortex slices incubated aerobically for 1 hr in the presence of protoveratrine or of ouabain, or in the absence of glucose, but not in the presence of L- or D-glutamate. Tetrodotoxin has similar effects. The barbiturates (amytal, pentothal), used at anesthetic concentrations that completely block potassium, or electrically stimulated brain respiration, have no such suppressing effect on Na^+ uptake. However, in calcium-free media and under conditions where brain respiration is being stimulated by the presence of protoveratrine or 30 mM KCl or 0·03 mM 2,4-dinitrophenol, the barbiturates exert significant depressions on both respiration and the uptake of Na^+. It is inferred that the effects of local anesthetics and of tetrodotoxin on the uptake of Na^+ into brain slices are due to their block of the generation of action potentials, whereas those of the barbiturates are due to their suppression of mitochondrial metabolism, causing release of mitochondrial Ca^{2+} and thereby resultant changes in the permeability of the cell membrane to Na^+ and K^+.

IT HAS been shown by Chan and Quastel[1] that the local anesthetics (cocaine, lidocaine, procaine) suppress the increased Na^+ uptake into brain cells that occurs when rat brain cortex slices, incubated aerobically in a physiological saline–glucose medium, are stimulated by application of electrical impulses, but that the barbiturates (amytal, pentothal), used at concentrations that completely block electrically stimulated respiration, have no such effect on Na^+ uptake. This demonstration was made both by direct measurements of uptake of $^{22}Na^+$ and indirectly by measuring the changes in the rates of cerebral oxidation of [1-^{14}C]acetate to $^{14}CO_2$, a process which is suppressed by Na^+ and stimulated by K^+, that take place in electrically stimulated brain slices in the presence of anesthetics.

We have made further studies of the effects of local anesthetics and tetrodotoxin and also of barbiturates on the increased uptake of Na^+, into incubated rat brain cortex slices, which occurs under a variety of conditions that are considered to lead to the generation of action potentials.[2]

The results of these experiments and their implication in our understanding of the mode of action of anesthetics are discussed below.

MATERIALS AND METHODS

Preparation of brain slices and incubation procedure

Adult rats of the Wistar strain, of either sex, weighing 150–200 g, were killed by stunning and dislocation of the neck. The brains were quickly removed and placed in Krebs–Ringer phosphate medium of the same composition as that in which they

were to be incubated subsequently. The medium was bubbled with 100% oxygen in a beaker surrounded by crushed ice. Brain cortex slices (60–80 mg), 0·3 mm thick, were cut from the cerebral hemispheres with a chilled Stadie–Riggs tissue slicer moistened with Ringer medium. Only the first slice from each of the dorsal and lateral surfaces was taken. The slices were weighed at once on a torsion balance and transferred to chilled Warburg manometric flasks containing a final volume of 3 ml of Krebs–Ringer phosphate glucose medium. Unless otherwise specified, the composition of Krebs–Ringer phosphate glucose medium was as follows: 128 mM NaCl, 5·0 mM KCl, 2·8 mM $CaCl_2$, 1·3 mM $MgSO_4$ and 10 mM Na_2HPO_4 (brought to pH 7·4 with 1·0 N HCl) and 10 mM glucose. The calcium-free medium consisted of this solution from which the calcium salt had been omitted. A solution of $^{22}NaCl$ (0·5 μc) was added to the incubation medium at the commencement of the experiment so that its concentration was 0·166 μc/ml. When potassium ions in the incubation medium were increased in concentration, potassium chloride was added to the normal medium. Sodium L- and D-sodium glutamate were added to the normal medium where specified, adjustments being made in the calculations of $^{22}Na^+$ uptake for the increased Na^+ content of the medium.

Unless otherwise stated, the final concentrations of anesthetics used were as follows: sodium amytal, 0·25 mM; pentothal, 0·5 mM; cocaine, 0·4 mM; lidocaine, 1·0 mM; and tetrodotoxin, 3 μM. Preliminary experiments showed that these concentrations of barbiturates and local anesthetics were unable to suppress the respiration, water uptake or sodium influx of unstimulated brain slices incubated in Krebs–Ringer phosphate-glucose medium. The concentrations of local anesthetics used were sufficient to suppress the increased sodium influx into the brain slices due to electrical stimulation, and the concentrations of amytal and pentothal used were sufficient to block the increased respiration of slices due to stimulation by electrical impulses or by increased potassium ion concentrations in this medium.

Measurements were made of the rates of oxygen uptake at 37° using the conventional Warburg manometric technique. Cups containing rolls of filter paper moistened with 0·2 ml of 20% KOH were placed in the center wells of the manometric vessels to absorb evolved carbon dioxide.

The vessels were gassed for 5 min with oxygen and 10 min were allowed for temperature equilibration at 37°. Incubation was usually conducted for 1 hr at 37°. The rates of oxygen consumption were uniformly linear under these experimental conditions.

Determination of water uptake

Immediately after the incubation, the slices were removed from the media and drained. They were spread without folding on an ice-cold clean glass surface and excess of fluid around the slices was absorbed with strips of filter paper. The slices were further drained by touching the glass surface several times until no cloud formed on the surface. The slices were then weighed accurately on a torsion balance. The entire procedure took less than 1 min. The difference between the weights of the slices before and after incubation gave a measure of the water uptake. This difference in milligrams multiplied by 100/A (where A was the initial wet weight of tissue in milligrams) was the amount of water in microliters taken up by the tissue per 100 mg initial wet weight.

Determination of ^{22}Na uptake

The brain slices, after final weighing, were quickly rinsed with 20 ml incubation medium (free from radioactivity), drained and homogenized in 3 ml of 5% (w/v) trichloroacetic acid with a Teflon homogenizer and left at room temperature for 2 hr. Control experiments showed no loss of tissue ^{22}Na due to the rinse. The homogenates were periodically mixed well in a Vortex–Genie shaker, centrifuged and their radioactivities were measured using 0·5-ml aliquots of the supernatants. The radioactivity of the medium was determined as follows: 0·4 ml of the incubation medium was added to 1·6 ml of 5% trichloroacetic acid in a centrifuge tube, mixed well and centrifuged. A 0·5-ml aliquot was added to 10 ml of scintillant. The scintillant consisted of equal volumes of dioxan, toluene and 95% (v/v) ethanol containing 1,4-bis-(5-phenyl-oxazol-2-yl) benzene (0·005%), 2,5-diphenyloxazole (0·5%) and naphthalene (8%).

The radioactivities were counted in a Nuclear-Chicago scintillation counter Mark I. The counting efficiency was corrected for each sample by means of the channels ratio technique. The usual counting efficiencies were between 21 and 23 per cent for ^{22}Na.

The amount of $^{22}Na^+$ taken up, in terms of microequivalents per gram initial wet wt. of tissue, was given by the expression:

$$\frac{\text{disintegrations per minute per gram initial wet wt.}}{\text{disintegrations per minute per milliliter of medium}} \times \text{microequivalents of } Na^+ \text{ per milliliter of medium}$$

In most of the experiments, the concentration of Na^+ in the incubation medium was 148 μ-equiv./ml.

The addition of $^{22}NaCl$ (0·166 μc/ml of medium), amounting to 0·07 nmole/ml, to the medium in which slices from rat brains were being incubated aerobically at 37° led to a rapid exchange between the labeled and unlabeled Na^+ in the slices. Almost 80 per cent of the maximum uptake of $^{22}Na^+$ took place within 4 min after the commencement of incubation. Within the incubation period of 1 hr, all the $^{22}Na^+$ exchanged with the unlabeled Na^+ in the slice.[2] Thus the content of $^{22}Na^+$ in the slice at the termination of the experiment, lasting 1 hr, gave a measure of the total Na^+ in the slice at that time. The method proved to be accurate, rapid and convenient.

Determination of potassium in tissue

Aliquots (1 ml) of the supernatant from the centrifuged tissue homogenate (3 ml) in 5% trichloroacetic acid were diluted each with 13 ml of glass-distilled water in polythene vials. These vials had been freed of inorganic ions by soaking overnight in 2% nitric acid in glass-distilled water, and rinsing with glass-distilled water to remove all traces of acid. They were dried over an infrared lamp. Potassium was assayed with the use of a Perkin–Elmer model 303 atomic absorption spectrophotometer. Standard solutions of potassium chloride were used for reference. Values were expressed as microequivalents of K^+ per gram initial wet wt. of tissue.

Chemicals

^{22}NaCl (69 mc/mg) was obtained from the Radio-Chemical Center, Amersham-Searle Corp., Toronto, Canada. It was diluted with water and stored frozen. Tetrodotoxin was purchased from the Sankyo Company Ltd., Tokyo. Protoveratrine maleate was the product of K & K Laboratories, Inc., New York, U.S.A. Sodium amytal was the product of Eli Lilly & Company, Toronto, Canada. Cocaine hydrochloride was obtained from British Drug Houses, Toronto, Canada, sodium pentothal from Abbott Laboratories, Montreal, Canada, and lidocaine (base) from Astra Pharmaceuticals. Most of the other chemicals were from Fisher Scientific Company, and were of A.C.S. certified grade.

Expression of results

Whereas formerly[1] the $^{22}Na^+$ concentration in the tissue slice was routinely corrected for the additional fluid taken up during incubation, the correction consisting of subtracting from the radioactivity accumulated in the brain slice that amount present in a volume of the medium equivalent to the amount of additional fluid taken up by the tissue slice, this correction has not been made in the present article. Instead, measurements of both the total ^{22}Na concentration in the brain slice and the amount of fluid uptake at the end of the incubation period are recorded. The reason for this procedure is that it has been shown that much of the water uptake in the brain slices is intracellular,[3,4] and that the process of water uptake may have a tetrodotoxin-sensitive component.[2] The value of this procedure is indicated below, where it will be seen that anesthetics may markedly affect the extent of fluid uptake in the incubated brain slices.

Reproducibility of results

All experiments recorded below were carried out at least four times. The means of these results and the standard deviations from the means are recorded.

RESULTS

Effects of amytal or pentothal on the Na^+ content, water uptake and respiration of rat brain cortex slices incubated under various conditions

Incubation conditions were chosen that are known to bring about significant increases of Na^+ uptake into rat brain cortex slices over those found when these are incubated aerobically in a physiological saline–glucose medium. Protoveratrine (5 μM) and ouabain (0·1 mM) bring about an increased uptake of Na^+, which is either wholly or partially suppressed by tetrodotoxin,[2] and which, it is inferred, bring about the generation of action potentials in the incubated brain slices.[2] Sodium L-glutamate and D-glutamate are well known to be excitatory amino acids and they both bring about a considerable uptake of Na^+ into the incubated tissue.[2,5–9] Results given in Table 1 show that, of all these substances, only protoveratrine brings about an appreciable increase in the rate of oxygen consumption, but that they all cause an increased water uptake in the incubated rat brain slices and an increased uptake of Na^+.

Amytal (0·25 mM), at a concentration approximately that present in the blood of rats under amytal anesthesia,[10] suppresses ($P < 0·01$) the increased rate of respiration

due to the addition of protoveratrine (5 μM), but diminishes neither the uptake of water nor that of Na^+. Pentothal (0·5 mM) behaves in a similar manner.

The increased uptake of Na^+ or of water into the incubated rat brain slices, due to ouabain, is not diminished by amytal (0·25 mM) or by pentothal (0·5 mM). Moreover, amytal (0·25 mM) exerts no suppressing action on the increased uptake of Na^+ or of water that occurs when sodium L-glutamate (5 mM) or sodium D-glutamate is added to the incubation medium (Table 1).

TABLE 1. EFFECTS OF SODIUM AMYTAL OR PENTOTHAL ON SODIUM CONTENT, WATER UPTAKE AND RESPIRATION OF RAT BRAIN CORTEX SLICES INCUBATED UNDER VARIOUS CONDITIONS*

Anesthetic present	Additions to the incubation medium	O_2 uptake (μmoles/100 mg initial wet wt.)	Water uptake (μl/100 mg initial wet wt.)	$^{22}Na^+$ content (μequiv./g initial wet wt.)
Nil	Nil	9·7 ± 0·2	19·0 ± 1·9	100 ± 6
	Protoveratrine (5 μM)	13·2 ± 1·0	25·0 ± 2·3	133 ± 6
	Ouabain (0·1 mM)	9·0 ± 0·5	40·0 ± 1·5	165 ± 4
	L-Glutamate (5 mM)	10·1 ± 0·2	38·1 ± 3·0	137 ± 4
	D-Glutamate (5 mM)	8·9 ± 0·6	48·0 ± 5·0	147 ± 4
Amytal (0·25 mM)	Nil	9·2 ± 1·0	22·4 ± 2·9	100 ± 7
	Protoveratrine (5 μM)	10·8 ± 0·9	35·0 ± 2·3	145 ± 5
	Ouabain (0·1 mM)	7·6 ± 0·1	37·0 ± 3·0	161 ± 2
	L-Glutamate (5 mM)	9·3 ± 0·5	38·0 ± 2·0	141 ± 1
	D-Glutamate (5 mM)	9·4 ± 0·2	50·5 ± 2·0	144 ± 7
Pentothal (0·5 mM)	Nil	9·8 ± 0·8	18·9 ± 1·6	99 ± 5
	Protoveratrine (5 μM)	6·4 ± 0·1	30·5 ± 3·0	137 ± 2
	Ouabain (0·1 mM)	7·6 ± 0·1	36·0 ± 2·0	165 ± 2

* Rat brain cortex slices were incubated aerobically at 37° for 1 hr in Krebs–Ringer phosphate medium containing 10 mM glucose and $^{22}Na^+$ (0·166 μc/ml medium). Anesthetics were present from the start of the experiments. Both L- and D-glutamates were added as their sodium salts.

Effects of tetrodotoxin, cocaine or lidocaine on the Na^+ content, water uptake and respiration of rat brain cortex slices incubated under various conditions

Tetrodotoxin (2 μM), as already reported,[2] blocks the increased Na^+ and water uptake of incubated rat brain cortex slices in the presence of protoveratrine (5 μM), but has little or no effect on the content of Na^+ or of water in the presence of L- or D-glutamate at the end of the 1-hr incubation period. Results are shown in Table 2. They indicate that both cocaine (0·4 mM) and lidocaine (1 mM), like tetrodotoxin, block the increased Na^+ and water uptake of incubated brain slices that occurs in the presence of protoveratrine ($P < 0·01$). Moreover, both cocaine and lidocaine, at the concentrations quoted, like tetrodotoxin, diminish the uptake of Na^+ that occurs in the presence of ouabain ($P < 0·01$). Cocaine, however, does not diminish the tissue content of Na^+ or of water found in the presence of L-glutamate or D-glutamate (Table 2). The local anesthetics suppress the increased respiration due to protoveratrine.

TABLE 2. EFFECTS OF TETRODOTOXIN, COCAINE OR LIDOCAINE ON SODIUM CONTENT, WATER UPTAKE AND RESPIRATION OF RAT BRAIN CORTEX SLICES INCUBATED UNDER VARIOUS CONDITIONS*

Anesthetic present	Additions to the incubation medium	O_2 uptake (μmoles/100 mg initial wet wt.)	Water uptake (μl/100 mg initial wet wt.)	$^{22}Na^+$ content (μequiv./g initial wet wt.)
Tetrodotoxin (3 μM)	Nil	9·7 ± 0·2	22·5 ± 1·0	102 ± 2
	Protoveratrine (5 μM)	11·0 ± 1·0	18·0 ± 1·0	110 ± 2
	Ouabain (0·1 mM)	10·2 ± 0·1	28·0 ± 0·5	141 ± 3
	L-Glutamate (5 μM)	9·9 ± 0·2	33·0 ± 2·0	134 ± 3
	D-Glutamate (5 mM)	8·5 ± 0·4	46·0 ± 5·0	142 ± 2
Cocaine (0·4 mM)	Nil	8·7 ± 0·3	23·0 ± 1·0	109 ± 3
	Protoveratrine (5 μM)	9·4 ± 0·2	19·0 ± 3·2	104 ± 4
	Ouabain (0·1 mM)	9·6 ± 0·3	37·0 ± 1·0	142 ± 7
	L-Glutamate (5 mM)	9·7 ± 0·3	37·8 ± 1·0	137 ± 7
	D-Glutamate (5 mM)	8·9 ± 0·2	52·4 ± 7·1	162 ± 6
Lidocaine (1 mM)	Nil	10·4 ± 0·3	13·8 ± 0·8	99 ± 2
	Protoveratrine (5 μM)	9·1 ± 0·4	18·0 ± 1·0	113 ± 1
	Ouabain (0·1 mM)	8·7 ± 0·9	34·0 ± 4·5	141 ± 6

* Conditions as in Table 1.

Effects of anesthetics on sodium content, water uptake and respiration of rat brain cortex slices incubated in glucose-free media

It has already been shown[2] that tetrodotoxin partially suppresses the increased uptake of Na^+ that takes place when rat brain cortex slices are incubated aerobically in a glucose-free saline medium. Experiments were therefore carried out to observe the effects of various anesthetics on this process. Results, recorded in Table 3, indicate that cocaine (0·4 mM) and lidocaine (1 mM) resemble tetrodotoxin (3 μM) in partially suppressing the Na^+ uptake that occurs in brain slices incubated in a glucose-free medium, but that amytal (0·25 mM) or pentothal (0·5 mM) has no such effect. No significant changes, by the anesthetics, of the water uptake found in the glucose-free medium are apparent, except for a decrease with tetrodotoxin, a phenomenon already described.[2]

TABLE 3. EFFECTS OF ANESTHETICS ON SODIUM CONTENT, WATER UPTAKE AND RESPIRATION OF RAT BRAIN CORTEX SLICES INCUBATED IN GLUCOSE-FREE MEDIA*

Anesthetic present	O_2 uptake (μmoles/100 mg initial wet wt.)	Water uptake (μl/100 mg initial wet wt.)	$^{22}Na^+$ content (μequiv./g initial wet wt.)
Nil	5·4 ± 0·5	37·0 ± 2·0	164 ± 5
Amytal (0·25 mM)	5·6 ± 1·1	41·1 ± 1·7	169 ± 4
Pentothal (0·5 mM)	6·1 ± 0·2	34·5 ± 2·5	158 ± 4
Tetrodotoxin (3 μM)	5·4 ± 1·0	28·0 ± 1·0	145 ± 5
Cocaine (0·4 mM)	5·2 ± 0·3	35·0 ± 2·0	146 ± 2
Lidocaine (1 mM)	5·1 ± 0·2	35·0 ± 2·0	129 ± 3

* Conditions as in Table 1, except that glucose was omitted from the incubation medium. No additions (apart from the anesthetic) were made to the incubation medium.

Effects of anesthetics on sodium content, water uptake and respiration of brain cortex slices incubated in calcium-deficient media

Although there is no suppression by barbiturates of Na^+ uptake into rat brain cortex slices incubated aerobically in calcium-containing media under conditions where local anesthetics are effective, it is found that, under certain conditions and only in calcium-deficient incubation media, barbiturates can suppress Na^+ influx into rat brain cortex slices.

Results obtained using calcium-deficient media are shown in Table 4. They indicate that when amytal (0·25 mM) or pentothal (0·5 mM) is added to rat brain cortex slices incubated aerobically in a calcium-free Krebs–Ringer phosphate medium, the barbiturate suppresses the raised rate of oxygen consumption that occurs under such conditions and that pentothal, but not amytal, brings about a small but significant reduction in the rate of entry of Na^+ into the incubated rat brain cortex slices. Even in the presence of 3 mM ethanedioxybis (ethylamine)tetra-acetate (EGTA), an effective chelator of Ca^{2+} which greatly reduces the small quantity of free Ca^{2+} that may still be present in the incubation medium,[11] amytal is still ineffective in reducing Na^+ uptake, though pentothal exerts a small suppressing effect.

Studies were then made of the effects of barbiturates added to calcium-free media where incubation conditions were such as to raise the respiratory metabolism of the

TABLE 4. EFFECTS OF ANESTHETICS ON SODIUM CONTENT, WATER UPTAKE AND RESPIRATION OF RAT BRAIN CORTEX SLICES INCUBATED IN CALCIUM-DEFICIENT MEDIA*

Additions to the incubation medium	Anesthetic present	O_2 uptake (μmoles/100 mg initial wet wt.)	Water uptake (μl/100 mg initial wet wt.)	$^{22}Na^+$ content (μequiv./g initial wet wt.)
Nil	Nil	12·0 ± 0·1	21·9 ± 2·5	137 ± 6
	Amytal	9·8 ± 0·4	20·8 ± 2·8	138 ± 4
	Pentothal	10·0 ± 0·5	16·2 ± 1·5	125 ± 3
KCl (30 mM)	Nil	17·1 ± 1·0	27·6 ± 3·0	143 ± 5
	Amytal	13·2 ± 0·5	17·6 ± 1·4	127 ± 3
	Pentothal	11·7 ± 0·2	8·9 ± 1·2	112 ± 2
	Tetrodotoxin (3 μM)	15·2 ± 0·2	11·8 ± 0·4	133 ± 2
	Cocaine (0·4 mM)	12·0 ± 0·2	15·7 ± 0·9	136 ± 3
	Lidocaine (1 mM)	15·0 ± 1·0	21·3 ± 0·7	140 ± 5
Protoveratrine (10 μM)	Nil	14·0 ± 0·5	29·7 ± 4·3	165 ± 5
	Amytal	11·5 ± 0·3	21·6 ± 2·7	148 ± 4
	Pentothal	11·9 ± 0·4	18·6 ± 1·2	145 ± 1
2,4-Dinitrophenol (0·03 mM)	Nil	17·2 ± 0·1	31·0 ± 3·0	181 ± 2
	Amytal	12·8 ± 0·3	32·5 ± 1·5	163 ± 7
	Pentothal	12·7 ± 0·5	27·7 ± 0·7	169 ± 1
2,4-Dinitrophenol (0·1 mM)	Nil	10·3 ± 0·3	37·0 ± 2·5	188 ± 7
	Amytal	8·5 ± 0·5	38·9 ± 4·0	188 ± 5
	Pentothal	9·0 ± 0·1	40·0 ± 1·0	183 ± 4

* Rat brain cortex slices were incubated aerobically at 37° for 1 hr in Krebs–Ringer phosphate media, devoid of Ca^{2+}, containing 3 mM EGTA, 10 mM glucose and $^{22}Na^+$ (0·166 μc/ml medium). Anesthetics were present from the start of the experiments. Sodium amytal, 0·25 mM; pentothal, 0·5 mM.

rat brain cortex slices. This was accomplished in three ways: (1) by increasing the K^+ concentration of the calcium-free medium to 35 m-equiv./l.;[1] (2) by the addition of protoveratrine (10 μM);[2] (3) by the addition of 2,4-dinitrophenol (0·03 mM). EGTA (3 mM) was added to such media to ensure as low a content of Ca^{2+} as possible.[11]

On the addition of 30 mM KCl to the calcium-free medium, the respiration of the brain slices is markedly augmented and under these circumstances both amytal and pentothal cause a significant ($P < 0·01$) reduction in the uptake of Na^+ into the incubated rat brain cortex slices. They suppress at the same time the stimulated respiration and the increased water uptake (Table 4). Pentothal (0·5 mM) is more effective than amytal (0·25 mM). It is of interest to note that neither cocaine nor lidocaine is as effective as pentothal or amytal in reducing Na^+ uptake under these conditions, affording another contrast between the effects of barbiturates and of local anesthetics on Na^+ movements in incubated rat brain slices. Tetrodotoxin brings about some reduction in Na^+ uptake, similar perhaps to the reported retardation of Na^+ uptake by tetrodotoxin that occurs when guinea pig brain slices[11] are incubated in a medium containing EDTA.

When protoveratrine (10 μM) is added to the calcium-free incubation medium, there is an acceleration of the rate of oxygen consumption which is completely blocked by amytal (0·25 mM) or pentothal (0·5 mM), and under these conditions both barbiturates suppress significantly ($P < 0·01$) the uptake of Na^+ into the incubated brain tissue (Table 4).

Addition of 0·03 mM 3,4-dinitrophenol greatly increases the rate of respiration of rat brain cortex slices incubated in a calcium-free, EGTA-containing, saline–glucose medium, and this increase is blocked by amytal (0·25 mM) or pentothal (0·5 mM) (Table 4). Results of a similar nature found with luminal or pentothal, but in calcium-containing medium, have already been reported.[12] Both amytal and pentothal, at the concentrations quoted, bring about a significant ($P < 0·01$) suppression of Na^+ uptake into brain cortex slices incubated in the calcium-free medium in the presence of 30 μM 2,4-dinitrophenol (Table 4).

TABLE 5. EFFECTS OF SODIUM AMYTAL OR PENTOTHAL ON THE Na^+ AND K^+ CONTENTS OF RAT BRAIN CORTEX SLICES INCUBATED AEROBICALLY IN CALCIUM-DEFICIENT MEDIA*

Additions to the incubation medium	Barbiturate present	K^+ content (μequiv./g initial wet wt.)
2,4-Dinitrophenol (0·03 mM)	Nil	21 ± 0·3
	Amytal (0·25 mM)	31 ± 0·5
	Pentothal (0·5 mM)	28 ± 0·4
KCl (30 mM)	Nil	58 ± 3
	Amytal (0·25 mM)	65 ± 0·5
	Pentothal (0·5 mM)	82 ± 1
Protoveratrine (10 μM)	Nil	19 ± 1
	Amytal (0·25 mM)	24 ± 0·1
	Pentothal (0·5 mM)	31 ± 0·5

On increasing the concentration of 2,4-dinitrophenol to 0·1 mM, at which concentration there is no acceleration of the rate of oxygen consumption, the barbiturates (amytal or pentothal) exert no suppression on the uptake of Na^+ into the incubated brain tissue (Table 4).

When the barbiturates bring about a diminished uptake of Na^+, there is an accompanying increased retention of K^+ in the incubated brain slices (Table 5). It is therefore evident that the barbiturates, at anesthetic concentrations and under specific incubation conditions, are able to modify the movements of both Na^+ and K^+ in brain tissue.

DISCUSSION

Tetrodotoxin, at low concentrations, suppresses the increased uptake of Na^+ that takes place when rat brain cortex slices are incubated aerobically in a physiological saline–glucose medium containing protoveratrine or ouabain,[2] or when electrical impulses are applied,[1] or when the incubation takes place in the absence of glucose.[2] It is inferred that under these incubation conditions action potentials are generated in rat brain cortex slices. It is known that local anesthetics resemble tetrodotoxin in suppressing action potentials[13,14] and reducing sodium conductance in axons.[15] Results given in this article show that the local anesthetics, cocaine (0·4 mM) and lidocaine (1 mM), have effects resembling that of tetrodotoxin in suppressing the increased accumulation of Na^+ occurring in incubated rat brain cortex slices. However, under these conditions where tetrodotoxin, cocaine or lidocaine suppresses the increased uptake of Na^+, the barbiturates, amytal (0·25 mM) and pentothal (0·5 mM), are ineffective. Thus, the behavior of the barbiturates, at anesthetic concentrations, differs from that of tetrodotoxin and local anesthetics in incubated brain tissue.

Nevertheless, both amytal (0·25 mM) and pentothal (0·5 mM) will suppress Na^+ uptake into rat brain cortex slices under certain conditions, i.e. incubation in calcium-deficient media, and with the addition of protoveratrine (10 μM) or of 0·03 mM 2,4-dinitrophenol or by the addition of KCl (30 mM) to augment respiratory metabolism. With raised K^+ in the incubation medium, neither cocaine nor lidocaine is as effective as amytal or pentothal, at the concentrations quoted, in suppressing the uptake of Na^+ (Table 4).

These results indicate the existence of at least two systems whereby Na^+ can accumulate in incubated brain slices. One is blocked by tetrodotoxin and local anesthetics and is presumably identical with the sodium current system associated with the generation of action potentials. This is not affected by barbiturates at anesthetic concentrations. The other system is manifested under conditions of calcium deficiency and augmented respiratory metabolism and is inhibited by barbiturates at anesthetic concentrations much more than by the local anesthetics investigated.

These results with the barbiturates become understandable when they are considered in relation to the known effects of amytal on the release of Ca^{2+} from cell mitochondria.[16] It has been shown[17–19] that mitochondria from a variety of tissues are able to accumulate Ca^{2+} either by a respiration-dependent process or by a process requiring the presence of ATP. This accumulation of Ca^{2+} by mitochondria is prevented by respiratory inhibitors, including amytal, and by a variety of uncoupling agents.[16] It is well known that amytal, in common with a variety of other barbiturates

and other depressants, suppresses the oxidation of substrates associated with NAD-linked dehydrogenases by inhibition of NADH oxidation.[20] Moreover, the suppression of mitochondrial respiration by amytal or pentothal occurs at concentrations equal to or less than 0·5 mM.[21] The concentration of ATP in rat brain cortex slices incubated aerobically for 1 hr in Krebs–Ringer media containing glucose is reduced 25 per cent by the presence of 0·5 mM amytal.[22]

It seems, therefore, reasonable to suggest that the mode of action of amytal or pentothal in suppressing Na^+ uptake by the brain slices in calcium-deficient media is connected with its release of Ca^{2+} from the brain cell mitochondria. The resultant increase of cytoplasmic Ca^{2+} may then reverse the membrane changes, in particular the increase of Na^+ permeability, which have come about as a result of Ca^{2+} deficiency in the incubation medium. A direct action of barbiturates on the membrane seems unlikely, in view of their lack of activity in the presence of a high concentration (0·1 mM) of 2,4-dinitrophenol. Such a conclusion is compatible with the view suggested by Godfraind *et al.*[23] that some anesthetics may act by causing a rise in cytoplasmic free Ca^{2+}, by suppression of mitochondrial metabolism resulting in a changed membrane permeability to K^+.

It is well known that sodium L-glutamate and D-glutamate, each at 5 mM, bring about with incubated rat brain cortex slices marked increases in Na^+ influx. The tissue contents of Na^+ are not significantly diminished at the end of the 1-hr incubation period by the presence of amytal (0·25 mM) or of cocaine (0·4 mM) or of tetrodotoxin (3 μM). It is possible that in the presence of the glutamates the influx of Na^+ is so rapid and extensive that it overcomes any effects of the anesthetics under the given experimental conditions.

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