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THE EFFECTS OF CERTAIN ANAESTHETIC AND ANTI-CONVULSANT DRUGS ON THE CSF POTASSIUM FLUXES OF THE DOG

J. HALLIDAY[1] & A.T.B. MOIR[2]

MRC Brain Metabolism Unit, Department of Pharmacology, Edinburgh University, 1 George Square, Edinburgh

1 A technique has been developed for open-ended perfusion of the cerebroventricular system of the unanaesthetized dog.

2 Perfusion with an artificial CSF solution containing inulin and ^{42}K allowed the potassium fluxes out of and into the CSF to be monitored over a period of 2 to 3 hours.

3 Sodium thiopentone and sodium pentobarbitone, in doses producing light anaesthesia, caused varying degrees of depression (up to 50%) in the CSF potassium fluxes, influx being consistently more affected than efflux. These effects are attributed to a decrease in the potassium exchange between extracellular and intracellular compartments in the brain.

4 Diazepam depressed both potassium fluxes by up to 10% while there was some evidence that diphenylhydantoin depressed only potassium influx.

5 Paraldehyde, in contrast to the other drugs, when given at a dose level sufficient to produce light anaesthesia, stimulated CSF potassium fluxes, particularly efflux.

Introduction

When the potassium concentration in the central nervous system is artificially raised, excitation and even convulsions may result (Meltzer, 1899; Koenigstein, 1951; Feldberg & Sherwood, 1957; John, Tschirgi & Wenzel, 1959; Nakajima, 1964). This phenomenon has been studied most closely by Zuckerman & Glaser (1968) who, working with the cat, used a localized, intraventricular perfusion technique to raise the potassium concentration in the fluid bathing the ventricular surface of the hippocampus. The resulting increase in excitability of the hippocampal region (i.e. lowered seizure threshold, spontaneous epileptiform activity, full clinical seizure) could be directly related to the degree to which the potassium concentration in the perfusion fluid was raised and to the time for which the perfusion was carried out.

It is now well established that the CSF and the brain extracellular fluid form a continuum (Wallace & Brodie, 1939, 1940; Rall, Oppelt & Patlak, 1962; Brightman 1965; Fencl, Miller & Pappenheimer, 1966; Davson & Welch, 1971). This has been confirmed for potassium by several elegant cerebroventricular perfusion studies with ^{42}K (Cserr, 1965; Bradbury & Davson, 1965; Katzman, Graziani, Kaplan & Escriva, 1965) and also by actual comparison of brain extracellular fluid and CSF (Cohen, Gerschenfeld & Kuffler, 1968). With respect to the excitability of the central nervous system, it is therefore significant that the potassium concentration in the CSF is homeostatically controlled (Bekaert & Demeester, 1951a, b; Dunker, 1957; Kemeny, Boidizsar & Pethes, 1961; Bradbury & Davson, 1965; Bradbury & Kleeman, 1967). It has been suggested that a localized breakdown in the potassium regulation in brain extracellular fluid may be a factor in epileptic phenomena (Green, 1964; Ovcharov, 1967; Zuckerman & Glaser, 1968; Fertziger & Ranck, 1970).

In the experiments to be described here, an examination has been made of the effects of certain anaesthetic and anticonvulsant drugs on the transport of potassium into and out of the CSF of the dog. The results suggest that certain of these drugs affect potassium transport between brain, blood and CSF, but do not demonstrate that an anaesthetic or anticonvulsant action is necessarily correlated with such effects. A

[1] Present address: Department of Pharmacology, King's College, London

[2] Present address: Scottish Home and Health Department, St Andrew's House, Edinburgh

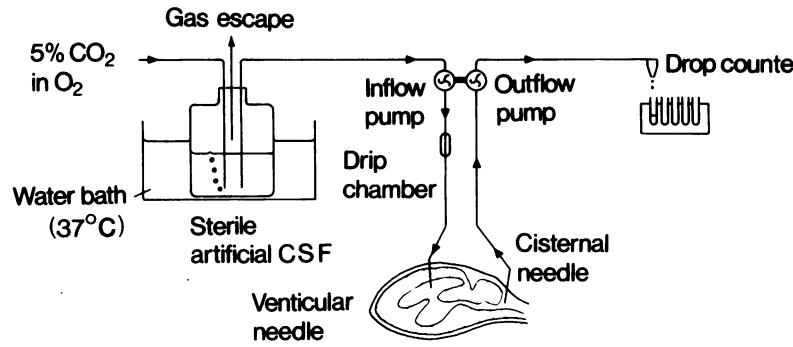

Fig. 1. Diagram of open cerebroventricular perfusion system. The inflow and outflow pumps are separate modules of the same multi-channel pump, both operating at the same flow rate.

preliminary communication concerned with some of the present data has already been given (Halliday & Moir, 1971).

Methods

The open cerebroventricular perfusion technique used in this study was a hybrid of the open perfusion system developed by Pappenheimer, Heisey & Jordan (1961) and the closed recirculatory system developed by Ashcroft, Dow & Moir, (1968).

Beagle dogs, of 12 to 16 kg weight, were first prepared for perfusion by the implantation, under fully aseptic conditions, of permanent guide tubes in the skull directed towards the lateral ventricles and the cisterna magna (Ashcroft *et al.*, 1968). After a 4-6 week recovery period the dogs were ready for perfusion.

In many open perfusion systems, flow through the system has been regulated by a single pump producing a constant rate of inflow, or by a constant pressure infusion mechanism situated on the inflow side of the circuit. The present system (Fig. 1) utilized an additional pump, situated between the cisternal outflow and the fraction collector. This extra pump was set at exactly the same flow rate as the pump on the inflow side of the circuit, both being equally calibrated modules of the same multi-channel pump (Watson-Marlow). This system allowed perfusion of the ventricular system of the fully conscious and unrestrained dog with minimal disturbance in the CSF pressure.

The artificial CSF perfused was a sterile salt solution containing (mM): Na^+ 151; K^+ 3.00; Mg^{++} 1.00; Ca^{++} 1.43; $H_2PO^-_4$ 0.58; HCO^-_3 25.6; Cl 132.67; and in addition inulin (20 mg/100 ml) and tracer amounts of ^{42}K(4-10 μCi/100 ml). Prior to, and throughout the duration of a perfusion, this perfusion fluid was maintained at 37°C and equilibrated with 5% CO_2 in O_2.

Once the infusion side of the circuit had been primed with perfusion fluid, the ventricular and cisternal needles were inserted percutaneously into the guide tubes (Ashcroft *et al.*, 1968). When a free flow of CSF had been established at both the ventricular and cisternal sites, the circuit was connected up and the perfusion pumps started. The rate of perfusion in all experiments was 300 ± 3 μl/minute. Perfusions were continued for 3-4 h with consecutive samples of perfusion effluent being collected in a fraction collector. The drugs or 5 ml 0.9% w/v NaCl solution were given as intravenous injections after 2 h of perfusion, thus allowing time for collection of both pre- and post-drug steady-state samples.

Initially samples were collected every 10 min, but as this volume of sample was not required to obtain accurate estimates after the first few experiments, the collection time was reduced to 4 minutes. In all experiments the pre-drug steady-state values were established on the basis of at least five consecutive steady-state samples, while post-drug samples were collected for a period of at least one hour. Samples collected in the 10 min period following drug administration were not included in the analysis.

^{42}K activity in the perfusate samples was estimated by Cerenkov counting in a liquid scintillation spectrometer (Nuclear Chicago Mark II), the counting efficiency being measured by a channels ratio method (Moir, 1971).

Inulin in the perfusate samples was estimated by the method described by Ashcroft *et al.* (1968).

Potassium in the perfusate samples was assayed by flame emission (Unicam SP. 90) against

standard potassium solutions made up in a solution of sodium chloride (160 mM).

Drugs

Sodium thiopentone (Pentothal) and sodium pentobarbitone (Nembutal), Abbott; diazepam (Valium), Roche; diphenylhydantoin (Epanutin), Parke-Davis; paraldehyde, Evans Medical.

Inulin, BDH; indol-3-yl acetic acid, Koch-Light; ^{42}K-potassium chloride solution, Radiochemical Centre.

Theory

The perfusion data were analysed in terms of a simple two-compartmental system, considering the CSF as one compartment and the brain and blood together as the other compartment.

Symbols

$\dot{V}$	flow rate	ml/min
C	concentration	mEq/ml
C*	radioactivity	d min^{-1} ml^{-1}
$\overline{C}$	mean concentration	mEq/ml
J	flux	mEq/min
J*	flux (as applied to a radioactive substance)	d min^{-1} ml^{-1}
N	net flux	mEq/min
N*	net flux (as applied to a radioactive substance)	d min^{-1} ml^{-1}

Subscripts have been applied as follows

i	perfusion inflow fluid
o	perfusion outflow fluid
f	freshly secreted CSF
a	fluid lost from system by bulk flow into subarachnoid space
v	ventricular compartment
b	composite brain and blood compartment

The use of v and b together implies a directional component from the first to the second.

Pappenheimer, Fencl, Heisey & Held (1965) have derived the equations describing net flux across the cellular barriers surrounding the perfused space.

$$N = \dot{V}iCi - \dot{V}oCo + \dot{V}fCf - \dot{V}aCo \quad (1)$$

$$= Jvb - Jbv \quad (2)$$

$$\therefore Jvb = \dot{V}iCi - \dot{V}oCo + \dot{V}fCf - \dot{V}aCo + Jbv \quad (3)$$

When ^{42}K is present in the inflow perfusion fluid J can be replaced by J* and C by C*. If one then assumes that J*bv and C*f can be taken as zero

$$J^*vb = \dot{V}iCi^* - \dot{V}oCo^* - \dot{V}aCo^* \quad (4)$$

Heisey, Held & Pappenheimer (1962) have shown that $\dot{V}a$ can be equated with the clearance of inulin from the perfusion system.

$$\therefore \dot{V}a = \frac{\dot{V}iCi - \dot{V}oCo}{Co} \text{ (where C refers to inulin)} \quad (5)$$

Assuming that ^{42}K is a perfect tracer for potassium, one can then calculate the steady-state efflux of potassium from the ventricles as

$$Jvb = \frac{\dot{V}iCi^* - (\dot{V}o + \dot{V}a)\,Co^*}{\text{mean specific activity of ventricular compartment}}$$

$$= \frac{\dot{V}iCi^* - (\dot{V}o + \dot{V}a)\,Co^*}{\left(\frac{Ci^* + Co^*}{Ci \quad Co}\right) \times 0.5} \quad (6)$$

Substitution of this value for Jvb in equation (3), assuming Cf to be 3.0 mM (Cserr, 1965), allows calculation of Jbv.

$$Jbv = \dot{V}oCo - \dot{V}iCi + \dot{V}aCo - 3.0\dot{V}f + Jvb \quad (7)$$

Results

The normal behaviour of the dogs was unchanged during the time of ventriculo-cisternal perfusion. The alterations in CSF pressure produced in the system by perfusion were of the same order as those produced by normal physiological variations (Figure 2).

From examination of data from control perfusion experiments in which no drug was administered, it was apparent that with the standard perfusion rate of 300 μl/min it took up to 1.5 h for the CSF system to become fully equilibrated with perfusion fluid. After this period a steady-state was reached in which levels of ^{42}K, potassium and inulin in the outflow perfusion fluid were stable. However, during this steady-state period, although the inulin clearance remained constant, both the computed potassium efflux and influx did display a very gradual decrease with time (Figure 3). In order to differentiate this effect from a superimposed, drug-induced change in the potassium fluxes, data from five control experiments were combined and best-fit quadratics computed to describe the apparent change in flux values with time during the course of a control perfusion. This curve could then be fitted to the initial pre-drug control phase of a drug experiment. By extrapolation it was possible to calculate hypothetical 'control' flux data for the post-drug phase of the experiment. Such control flux values were calculated for all times at which actual post-drug values were available, and the difference between the two was considered to

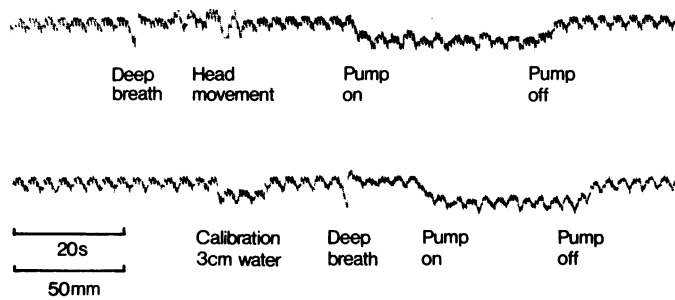

Fig. 2 Effect of perfusion on pressure within a dog's CSF system. At the point marked 'Calibration 3 cm water' the trace was offset by an amount equivalent to a 3 cm water decrease in cisternal pressure.

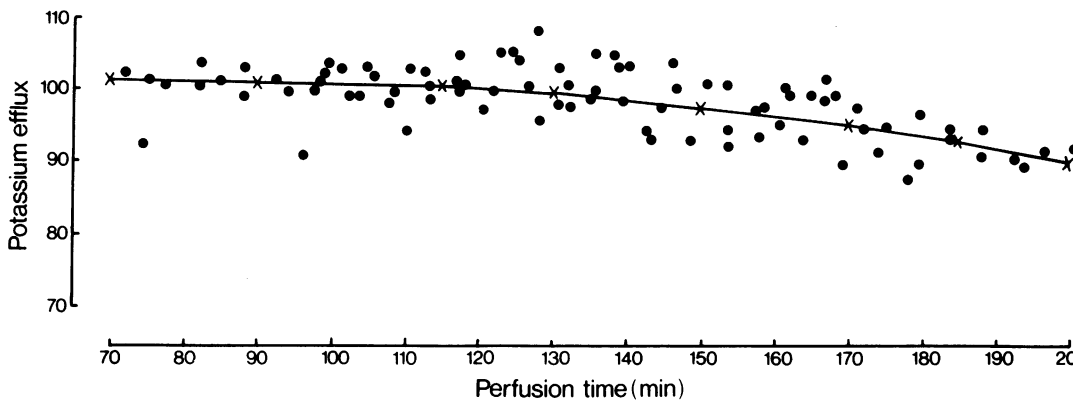

Fig. 3 Composite graph showing CSF potassium efflux data from five control experiments. The efflux data for each experiment has been expressed as a percentage of the mean efflux in that experiment during the time period 90-120 minutes. $y = 96.3 + 0.126\,r - 0.008r^2$.

reflect the drug-induced change in flux values. Comparing hypothetical control values and actual post-drug values as paired data, with respect to the time course of the perfusion, allowed a paired t-test approach to determining the significance of the drug-induced effect.

Barbiturate Anaesthetics

Sodium pentobarbitone and sodium thiopentone were given intravenously to produce a level of light anaesthesia in which the corneal reflex was present, but in which the response to a painful pressure stimulus applied between the toes was almost completely abolished. The dose required with both drugs was between 20 and 24 mg/kg, though in the case of thiopentone, supplementary doses were necessary to maintain the required state of anaesthesia.

There was a significant reduction ($P < 0.01$) in potassium efflux in 5 out of the 8 experiments (Fig. 4) while the reduction in potassium influx was significant ($P < 0.01$) in 6 out of the 8 experiments. In the 6 experiments where there were significant changes, a paired t-test analysis on the post-drug samples comparing depression of influx with depression of efflux showed influx to be significantly ($P < 0.01$) more depressed than efflux.

Diazepam

Diazepam (5 mg/ml solution) was administered by slow intravenous injection. The dose given, namely 20 mg (i.e. 1.3 mg/kg) is twice the initial dose used in man to induce brief anaesthesia or to control status epilepticus. Before the injection was completed, the dog usually displayed signs of muscle relaxation and became slightly ataxic, but anaesthesia was not induced.

Out of the 3 diazepam experiments, 2 showed a

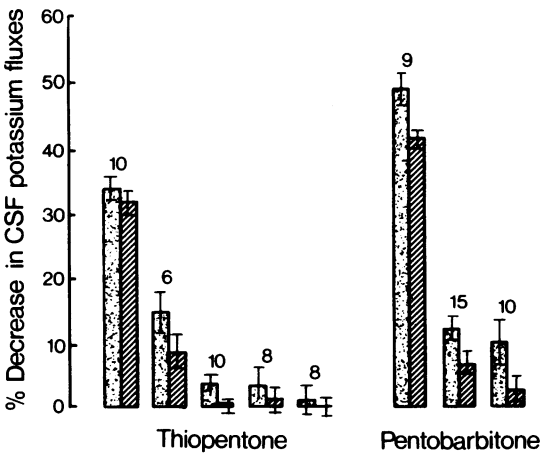

Fig. 4 Effect of barbiturate anaesthetics on CSF potassium fluxes. Each influx (stippled columns)/ efflux (hatched columns) pair refers to one perfusion experiment, being the mean drug-induced flux change. Vertical bars indicate standard errors. Number of samples shown in above columns. These changes, which were calculated from samples collected during the post-drug phase of a perfusion, have been expressed as percentages of the steady-state fluxes during the pre-drug phase of the perfusion.

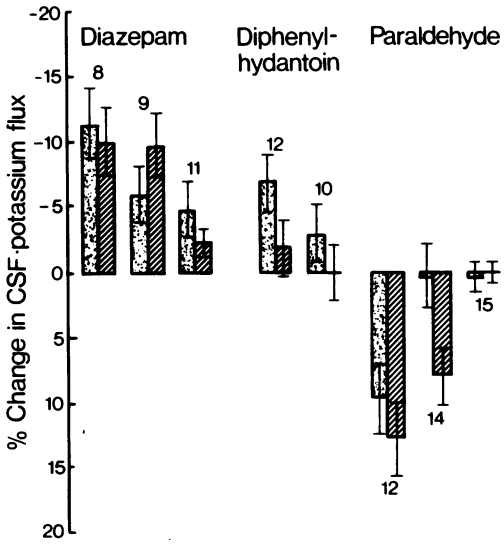

Fig. 5 Effect of selected anticonvulsants on CSF potassium fluxes. Each influx (stippled columns)/ efflux (hatched columns) pair refers to one perfusion experiment, being the mean drug-induced flux change (–, decrease; +, increase). These changes, which were calculated from samples collected during the post-drug phase of a perfusion, have been expressed as percentages of the steady-state fluxes during the pre-drug phase of the perfusion. Vertical bars indicate standard errors. Number of samples shown above columns.

significant reduction ($P < 0.01$) in potassium efflux (Fig. 5) while there was a significant reduction ($P < 0.02$) in potassium influx in all 3 experiments. The flux depressions varied from 3 to 11% and there was no significant difference between the effects on efflux and influx.

Diphenylhydantoin

Diphenylhydantoin was administered intravenously in a dose of 5 mg/kg. No behavioural changes or physical signs could be detected after this dose. In the 2 experiments there was no significant change in potassium efflux (Fig. 5) but there were reductions of 2 and 7% in potassium influx, these changes being significant at the probability levels of $P < 0.05$ and $P < 0.01$ respectively.

Paraldehyde

Paraldehyde (2.5 to 4.0 ml/kg) was administered by intramuscular injection. Over the 15 min following the injection, the dogs lapsed into a state of anaesthesia in which they no longer responded to strong interdigital pressure. This drug, in contrast to the others studied, caused either no change or an increase in potassium fluxes (Figure 5). In one experiment there were significant ($P < 0.01$) increases in potassium efflux and influx of 13 and 10%, respectively. In the second experiment there was a significant ($P < 0.01$) increase of 8% in efflux and no change in influx while in the third experiment there was no change in either flux.

Discussion

Although in this study we have not discriminated between the brain and blood compartments, present knowledge of brain and CSF potassium control mechanisms may allow us to infer the relative contribution of each of these two compartments to the measured potassium fluxes. From the early studies of Cserr (1965), Bradbury & Davson (1965) and Katzman *et al.* (1965), it is known that most of the potassium leaving the CSF can subsequently be recovered from the brain. Further studies by Bradbury & Stulcova (1970) have shown that the other fraction of the potassium leaving the CSF passes into the blood

by way of a transport mechanism thought to be a sodium/potassium pump, the potassium being directed towards the blood. This transport mechanism, which could be in the brain capillary (Bradbury, Segal & Wilson, 1972), is a major factor in homeostatic control of potassium levels, since its activity is low at normal CSF potassium levels (10-20% of normal CSF potassium efflux), but increases rapidly and linearly as CSF potassium levels are increased up to 10 mM. At normal levels of CSF potassium, therefore, about 80% of the CSF potassium efflux is due to simple molecular exchange between CSF potassium and brain potassium. This exchange will be related to neuronal activity which brings about rapid mixing between potassium in intracellular and extracellular compartments.

Direct influx from blood accounts for only a small portion of the total potassium influx into CSF (Cserr, 1965). Bradbury & Kleeman (1967) have postulated that potassium may enter the CSF from blood firstly by way of a saturated carrier mechanism and secondly by some form of transport in which the amount entering CSF is proportional to the level of potassium in the plasma. Most of the potassium entering the CSF is derived from brain and, assuming steady-state conditions with respect to total brain potassium, this would follow from the postulate of a large exchange between brain and CSF potassium.

The observed effects on potassium fluxes have been interpreted on the basis of these known physiological mechanisms.

Variability was a feature of the response to the barbiturate anaesthetics. In some experiments there was a reduction of up to 50% in CSF potassium fluxes, while in others there was no significant change. When significant changes were seen, the reduction in influx and efflux were always of a similar order of magnitude, although influx was consistently more depressed than efflux. Three findings seem to be against a barbiturate-induced reduction in active transport of potassium from the CSF to the blood. Firstly, on no occasion did a reduction in efflux occur in isolation from a similar reduction in influx. Secondly, the magnitude of the inhibition which could occur, in several cases, amounted to a greater reduction in efflux than could be achieved even by complete inhibition of the system actively transporting potassium into the blood. Finally, the existence and nature of the system actively transporting potassium from the CSF into the blood was first elucidated and characterized in experiments involving the use of animals under deep barbiturate anaesthesia (Bradbury & Stulcova, 1970).

Barbiturate anaesthesia is unlikely to affect the passive diffusion of potassium into the brain extracellular space, therefore it would seem to be causing a reduction in the exchange between brain intracellular and extracellular potassium. Such an explanation would account for a corresponding reduction in potassium influx. It would also be entirely consistent with the depressant effect of barbiturates on neuronal activity. It seems probable, therefore, that the potassium flux values for anaesthetized animals quoted by other workers (Bradbury & Davson, 1965; Cserr, 1965; Katzman *et al.*, 1965; Bradbury & Stulcova, 1970) may enderestimate the extent of brain/CSF potassium exchange which occurs in conscious animals.

Accepting this explanation for the reduction in potassium efflux accounts for a similar reduction in influx, but still leaves a further observed reduction in influx to be explained. Assuming the potassium content of brain cells remains constant, it would seem that there is some inhibition of the transport of potassium from blood into the CSF though whether the postulated saturated carrier mechanism or the unsaturated, concentration-dependent process is being affected cannot be determined from the present experiments. There is some support for the latter possibility in that barbiturate anaesthesia lowers plasma potassium levels (Bradbury & Davson, 1965).

Diazepam produced a slight decrease in both efflux and influx of potassium. The fact that efflux and indlux were reduced to a similar extent suggests that the drug might be inhibiting a single mechanism affecting both efflux and influx. The likeliest candidate would seem to be the process of exchange between brain intracellular and brain extracellular potassium.

Diphenylhydantoin had no effect on potassium efflux from the CSF but there was evidence that it had a slight depressant effect on potassium influx. If this were true, it would contrast with a preliminary report by Woodbury & Kemp (1971) that diphenylhydantoin increases potassium efflux from the CSF. However, as both mechanisms would lead to a reduced CSF potassium concentration these findings may not be as divergent as they at first seem. The use of anaesthetized as against unanaesthetized animals might also explain the anomaly. Woodbury & Kemp (1971) have hypothesized that the anticonvulsant action of diphenylhydantoin is due to its stabilizing effect on excitable membranes by increasing sodium transport out of the cell, thus providing a hyperpolarized membrane. This is supported by their own and Festoff & Appel's finding (1968) that diphenylhydantoin stimulates sodium-potassium ATPase activity. However, Izquierdo & Nasello (1970) have suggested that diphenylhydantoin may antagonize post-tetanic

potentiation and seizure activity by an action on potassium transport. It would therefore be premature to assume from the results of our experiments that diphenylhydantoin does not affect potassium in the brain.

Paraldehyde was unique among the drugs studied in that it appeared to increase potassium efflux from the CSF in 2 out of 3 cases. In one of these 2 cases the influx was not affected, but in the other, it also was increased. The stimulation of CSF potassium fluxes contrasted with the drug's obvious anaesthetic action. The drug was similar to the barbiturates in terms of anaesthetic effects, but dissimilar in its effects on CSF potassium fluxes. This was surprising, considering that Thesleff (1956) has reported that these drugs have very similar effects on the ionic permeability of muscle excitable membrane. However, very little is known about the mechanism of action of paraldehyde. It is possible, indeed, that the stimulant effect on potassium fluxes was due to a metabolite of the drug, such as acetaldehyde.

In conclusion then, the animal techniques and the analytical treatment of results which have been evolved provide, for the first time, a method of monitoring CSF fluxes of potassium and potentially other substances, over a period of 2 to 3 hours. From the limited number of drug experiments carried out it has been shown that barbiturate anaesthetics and diazepam can depress potassium fluxes both into and out of the CSF, influx being consistently more depressed than efflux. A decrease in the exchange of potassium between brain intracellular and extracellular compartments is thought to be largely responsible for these changes. There is some evidence that paraldehyde stimulates potassium exchange between CSF and brain (at the same time as it produces anaesthesia) and that diphenylhydantoin may decrease the influx of potassium into the CSF. These results indicate that anaesthetic and anticonvulsant drugs may affect potassium transport between brain, blood and CSF, but do not demonstrate that anaesthesia or an anticonvulsant action is necessarily correlated with such an effect.

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