Diphenylhydantoin and Potassium Transport in Isolated Nerve Terminals

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ABSTRACT The antiepileptic action of diphenylhydantoin (DPH) has been explained by two different theories: (*a*) that DPH stimulates the Na-K pump; (*b*) that DPH specifically blocks the passive translocation of sodium. Since electrophysiological experiments have recently suggested abnormal synaptic mechanisms as the basis for epileptogenic discharges, the action of DPH on K transport within synaptic terminals isolated from "normal" rat brain cortex was examined directly. A rapid filtration technique was used to assess in vitro potassium transport within synaptosomes. In vivo DPH did not significantly change endogenous K content within synaptosomes. With sodium (50 mM) and potassium (10 mM) concentrations optimal for Na-K pump activity, in vivo and in vitro DPH (10^{-4} M) had minimal or no effects on total K uptake. DPH stimulated potassium uptake within synaptosomes under two situations: (*a*) at high sodium (50–100 mM) and low potassium (less than 2 mM) concentrations; (*b*) when synaptosomes were incubated with ouabain (10^{-4} M) 50 mM Na and 10 mM K. In both situations, K was leaking out of synaptic terminals and the enhancement in net K uptake roughly corresponded to the ouabain inhibitable segment. In the absence of ouabain, the stimulatory effects of DPH were not observed when K was 2 mM or higher and when Na was 10 mM or lower. The stimulatory effects of in vitro DPH appeared over a range of concentrations from 10^{-4} to 10^{-10} M while single intraperitoneal injections of DPH had to be administered for 2 days before its effects were observed on synaptosomal K transport. The present data provided direct evidence for DPH stimulation of active potassium transport within synaptosomes under ionic conditions simulating the depolarized state. At other ionic conditions, DPH had inhibitory or no effects on K uptake. Although the results do not specify whether the effects of DPH on the Na-K pump are direct or indirect, they suggest that the action of DPH depends upon the state of the membrane and the specific ionic environment.

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INTRODUCTION

Studies of the mechanism of action of diphenylhydantoin sodium (DPH)[1] have centered on the cation transport systems of excitable membranes of the cerebral cortex (1, 3). In whole cerebral cortical tissues, DPH prevented intracellular increase of Na caused by maximal electroshock seizures and increased the turnover of Na^{22} (1). Because of the close link established between membrane-oriented sodium-potassium-adenosinetriphosphatase (Na-K-ATPase) and cation transport (2), synaptosomal Na-K-ATPase was previously studied in an attempt to explain the Na extruding properties of DPH. DPH stimulated synaptosomal Na-K-ATPase activity under conditions of high Na (100 mM) and low K (0.2 mM) while it had minimal or no effects on enzyme activity with 50 mM Na and 10 mM K (3). On the basis of these experiments, it was suggested that the anticonvulsant property of DPH may be related to its ability to enhance Na-K-ATPase activity and increase Na efflux during depolarized states. This suggestion was based on several assumptions: (*a*) that synaptosome Na-K-ATPase is involved in the active transport of K and Na; (*b*) that ion transport within synaptosomes is altered in convulsive states; and (*c*) that DPH reverses such alterations.

The present experiments directly examine K transport within synaptosomes isolated from "normal" rat cerebral cortex and investigate the action of DPH on such transport in vitro. In vivo and in vitro DPH stimulated active K transport within synaptosomes with 50–100 mM Na and less than 2 mM K while it reversed

[1] *Abbreviations used in this paper:* DPH, diphenylhydantoin; Na-K-ATPase, sodium-potassium-adenosinetriphosphatase.

ouabain inhibition of K transport with 50 mM Na and 10 mM K.

METHODS

Ficoll was obtained from Pharmacia Laboratories Inc., ouabain from Sigma Biochemicals, cesium chloride from Fisher Scientific Co, and diphenylhydantoin sodium from Parke Davis & Company.

Preparation of synaptosomes. Synaptosomes were prepared from 18 to 26-day old rat brain cortices (4). Synaptosomal fractions were kept in 0.32 M sucrose and used as soon as possible. The maximum number of hours elapsed between the time of isolation and the actual experimentation was 2 hr. Synaptosomes were identified as enriched populations of nerve endings by electron microscopy and enzyme-specific activity. The average yield of synaptosomal protein was approximately 2–3 mg/g wet weight of cerebral cortex.

Incubations and assessments of potassium accumulation. Incubations were performed at 23°C in 2 cc containing 0.033 M of highly purified tris chloride buffer (pH 7.5) 0.1 M sucrose, 2.8–3.2 mg of synaptosomal protein, and varying concentrations of Na and K. When sodium chloride was omitted from the medium, choline chloride in equimolar concentrations was added. During assessment of DPH effects in vitro, diphenylhydantoin prepared daily, was added in specified concentrations to the reaction mixture. In all experiments, synaptosomal proteins were added at the start of incubation. Concentrations of 10^{-4} M–10^{-10} M DPH did not alter the pH of the incubation mixture. During in vivo studies, DPH was administered intraperitoneally as a single daily dose (40 mg/kg body weight) for a specified number of days. The animals were sacrificed 24 hr after the last intraperitoneal dose.

The potassium content of synaptosomes was assessed by a rapid filtration technique (4). Samples (0.2 cc) were removed from the reaction mixture at appropriate times, pipetted into 5.0 cc of ice-cold 0.25 M sucrose, rapidly mixed by inversion, and immediately poured on a moist Millipore filter (Millipore Corp.), mounted on a suction flask. The filters were 25 mm in diameter and 0.45 μpore size. The mixture of sample and sucrose were suctioned through the filter until a minimal layer of fluid remained above the filter. The filters were then washed with an additional 25 cc of 0.25 M sucrose and suctioned until dry. The cations were extracted from the filter by addition of 2 cc of 1% HNO_3 containing 4 mM cesium chloride. The K content of the 2 cc extract was measured in a Perkin-Elmer atomic absorption spectrophotometer (Perkin-Elmer Corp.). Proteins were determined by the method of Lowry, Rosebrough, Farr, and Randall (5) and the K content was expressed in micromoles of K per milligram protein of synaptosomes.

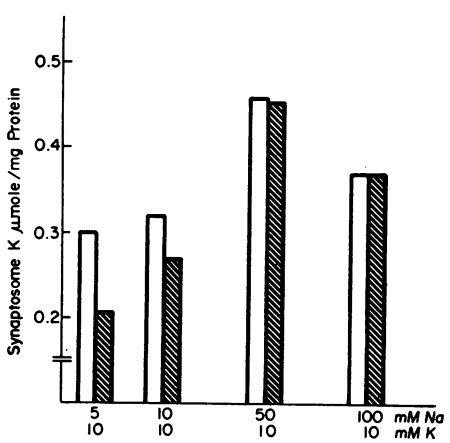

FIGURE 1 The effects of DPH on K accumulation within synaptosomes at varying Na concentrations. Synaptosomes were incubated and processed as described in Methods. Potassium (10 mM) and varying Na concentrations were added with (striped bars) and without □ DPH (10^{-4} M). The total height of the bars represent synaptosomal K content at 16 min incubations. Osmolarity was held constant by addition of varying concentrations of chloine chloride. The four experiments represented above were from one synaptosomal preparation. Similar results were obtained in four other synaptosome preparations.

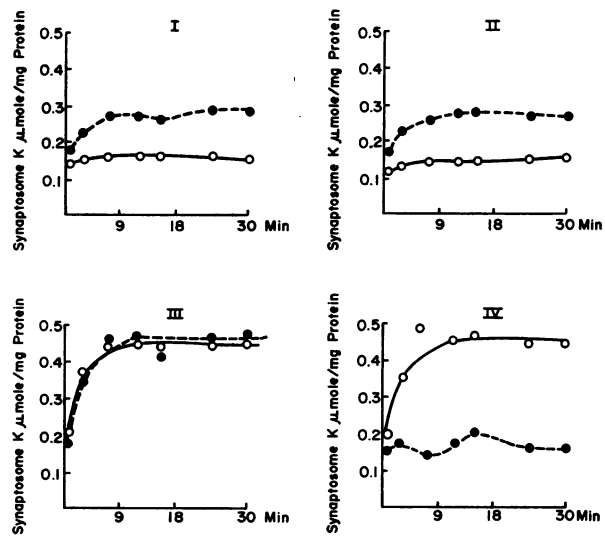

FIGURE 2 The effects of DPH on K accumulation in the absence and presence of ouabain. In Panels I and II, 5 mM Na and 10 mM K are present in the standard reaction mixtures. —●—●—●—●— in Panel I represent control synaptosomes and o—o—o are synaptosomes treated with DPH in vitro at 10^{-4} M. In Panel II, ouabain (10^{-4} M) is present in ●—●—● while DPH (10^{-4} M) and ouabain (10^{-4} M) are present in o—o—o. In Panels III and IV, 50 mM Na and 10 mM K are present in the incubations. ●—●—● in Panel III represent control synaptosomes and o—o—o—o DPH (10^{-4} M) treated synaptosomes. In Panel IV, ouabain has been incubated with synaptosomes of ●—●—●; DPH is present with ouabain in o—o—o.

The endogenous or base line K content of synaptosomes was measured immediately after isolation of synaptosomes. The incubation mixture without Na or K was used, and synaptosomes were added last. The test tubes were gently agitated twice and three 0.2 cc samples were immediately pipetted into three separate 5 cc-volume test tubes containing ice-cold 0.25 M sucrose. Subsequent steps were identical to those utilized in the filtration technique for in vitro K uptake experiments described above. The arithmetic mean of these three separate determinations were taken to represent endogenous synaptosomal K content.

The estimates of K content per milligrams of synaptosome protein were variable from preparation to preparation. Ac-

cumulation experiments in control and DPH-treated synaptosomes were performed from identical preparations making comparisons valid and reproducible. In DPH-treated preparations initial values at ½ min points were not consistently lower during inhibition or consistently higher during stimulation; attempts at calculating rates of K accumulation, therefore produced inconsistent results. The per cent stimulation or inhibition in these experiments were calculated by comparing 16 min equilibrium points in control and DPH experiments. Results were essentially similar when 8 and 30 min incubation points were compared.

RESULTS

In vitro experiments. Previous experiments on K accumulation within synaptosomes have demonstrated time dependent K uptake to be optimal with 50 mM Na and 10 mM K in the external medium. At these concentrations, ouabain produced an average 54% inhibition of net K accumulation (4).

The effects of diphenylhydantoin (10^{-4} M) on net K accumulation within synaptosomes were therefore initially determined with optimal K (10 mM) and varying Na concentrations. DPH inhibited K uptake 31% with 5 mM Na and 17% with 10 mM Na (Fig. 1). At 5 mM Na and 10 mM K, K accumulation was relatively insensitive to ouabain and DPH induced inhibition of net K uptake occurred even in the presence of ouabain. Hence, while DPH effects on the ouabain sensitive K accumulation could not be assessed under these ionic conditions, ouabain insensitive K uptake was inhibited (Fig. 2). At higher Na concentrations (50 mM and 100 mM), no significant effects on total K uptake was evident with DPH. However, ouabain inhibition of K transport which is optimum at these ionic conditions (4) were reversed by DPH (Fig. 2).

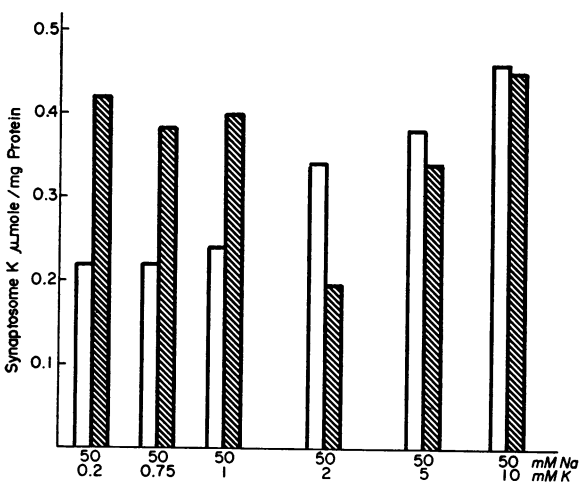

FIGURE 3 DPH effects on net K accumulation at varying K concentrations. Sodium (50 mM) and varying K concentrations are present in the reaction mixtures with (striped bars) and without □ DPH (10^{-4} M). Values obtained represent synaptosomal K content at 16 min incubations. Five experiments with one synaptosome preparation is represented. The total osmolarity was held constant by addition of varying concentrations of choline chloride. Similar results were obtained in three other synaptosome preparations.

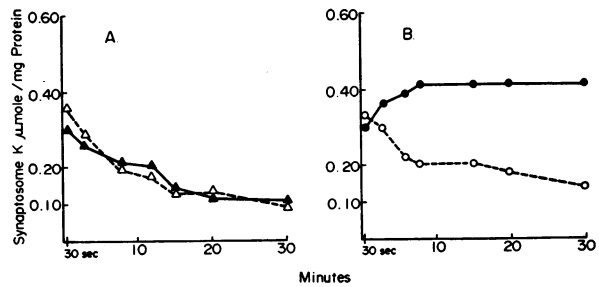

FIGURE 4 Stimulation by DPH of K accumulation within synaptosomes at 50 mM Na and 0.2 mM K. Incubations were as described in Methods. In A, ▲—▲—▲ represents control synaptosomes and △—△—△ synaptosomes treated with ouabain (10^{-4} M). In B, ●—●—● represents synaptosomes incubated with DPH (10^{-4} M) and ○—○—○ synaptosomes in reaction mixtures with both DPH (10^{-4} M) and ouabain (10^{-4} M).

Potassium concentrations were then varied at less than and up to 10 mM while Na was maintained at optimal concentration (50 mM). DPH stimulated K accumulation 72% with 0.2 mM K, 68% with 0.75 mM K, 70% with 1 mM K (Fig. 3). DPH inhibited net K accumulation at 2 mM K (42%) and at 5 mM K (9%). At 10 mM K, DPH produced no significant effects on total K accumulation (Fig. 3).

The stimulatory effects of DPH were best appreciated when K accumulation was studied as a function of time. At 50 mM Na and 0.2 mM K in the external medium, no K uptake was evident; in fact the K content of synaptosomes gradually declined during the first 9 min (from 0.36 to 0.21 μmoles K/mg protein). With the addition of DPH (10^{-4} M), the intrasynaptosomal K increased during the first 9 min from 0.3 to 0.4 μmoles K/mg protein (Fig. 4). During the subsequent 20 min, K content remained unchanged at 0.4 μmoles/mg protein. In seven separate synaptosomal preparations, DPH stimulated K accumulation by an average 91% with 50 mM Na and 0.2 mM K in the external medium (Table I). With no K and 50 mM sodium, DPH had no significant effects. However, as stated above at K concentrations of 0.75 mM and 1 mM, DPH stimulated K uptake. At all concentrations of high Na and low K, the stimulatory effects of DPH could be reversed by ouabain (10^{-4} M) (Fig. 4).

The K concentration was maintained at 0.2 mM and Na concentrations were varied from 0 to 100 mM. With these conditions, DPH was found to stimulate synaptosomal K accumulation only in the presence of high Na concentration. DPH had no effects in the absence of Na while net K accumulation and ouabain inhibition of K

TABLE I

The Effects of Diphenylhydantoin on Net K Accumulation at 50 mM Na and 0.2 mM K

Experiment	Control 0.5 min	Control 8 min	Control 16 min	DPH 0.5 min	DPH 8 min	DPH 16 min	Stimulation 16 min
	μmoles K/mg protein						%
1	0.36	0.20	0.20	0.30	0.46	0.48	140
2	0.30	0.18	0.16	0.15	0.32	0.33	105.7
3	0.29	0.23	0.23	0.20	0.45	0.46	102.2
4	0.34	0.18	0.17	0.18	0.33	0.33	94.0
5	0.36	0.21	0.16	0.30	0.40	0.40	82.0
6	0.36	0.30	0.30	0.30	0.48	0.48	60.7
7	0.36	0.31	0.31	0.26	0.47	0.48	53.2
					Mean ±SD		91.0 ±29.3

Reaction mixtures were incubated as described in Methods. Seven experiments with seven different synaptosomal preparations are represented.

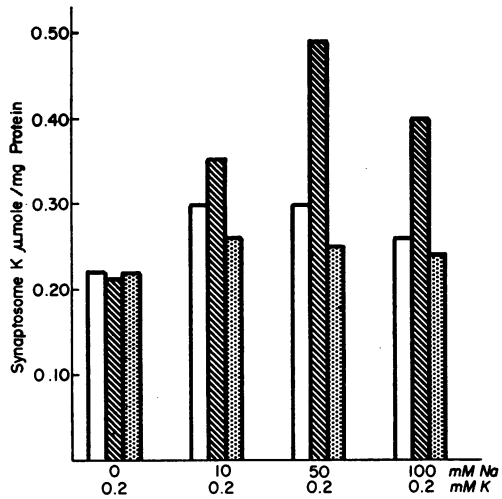

FIGURE 5 The effects of Na concentration on DPH enhanced K accumulation. The blocks represent K content at 16 min incubations in control □ and DPH (10^{-4} M) treated (striped bars) synaptosomes. Ouabain (10^{-4} M) effects on DPH treated synaptosomes are represented by stippled bars. Osmolarity was held constant with choline chloride addition. The above experiments represent one single synaptosome preparation. Two other separate synaptosome preparations showed similar effects of Na.

transport were enhanced 83% with 50 mM Na, 44% with 100 mM Na, 13% with 10 mM Na (Fig. 5).

Previous studies on synaptosomal Na-K activated ATPase suggested a 50:1 Na-K ratio as critical in determining the stimulatory effects of DPH (3); a similar relation was investigated with respect to K transport within synaptosomes. With concentrations in the external mediums of 10 mM Na and 0.2 mM K (Table II) (Fig. 5), as well as 20 mM Na and 0.4 mM K (Table II), minimal stimulation or no effects were observed. At 100 mM Na and 2 mM K, K uptake decreased 20%. Hence, a 50:1 Na-K ratio was not essential for DPH stimulation of net K accumulation. As shown by the above experiments (Fig. 5) (Table II), what appears optimal are a Na concentration of 50 mM and a K concentration less than 2 mM. The 50:1 ratio did not appear as meaningful as the actual levels of Na and K.

Stimulatory effects at 50 mM Na and 0.2 mM K appeared over a range of DPH concentrations. Total K accumulation was enhanced 77% with 10^{-4} M, 38.5% with 10^{-6} M and 23% with 10^{-8} M, while net K uptake was unaltered with 10^{10} M (Fig. 6). At 10^{-4} M to 10^{-8} M, ouabain inhibition was enhanced to approximately the same extent total K accumulation was stimulated by DPH. At 10^{-10} M, ouabain inhibition increased 36% although no apparent change was observed in total K

TABLE II

Na/K Ratio and the Effects of DPH on Net K Accumulation

Preparation	Na	K	Control 0.5 min	Control 8 min	Control 16 min	DPH 0.5 min	DPH 8 min	DPH 16 min	Stimulation (+) or inhibition (−) 16 min
	(mM)	(mM)	*μmoles K/mg protein*						%
1	10	0.2	0.20	0.15	0.15	0.20	0.16	0.17	+10
	20	0.4	0.18	0.17	0.17	0.18	0.17	0.18	+3.7
	50	1	0.12	0.20	0.20	0.12	0.36	0.35	+75
	100	2	0.12	0.30	0.30	0.15	0.25	0.25	−16.66
2	10	0.2	0.22	0.14	0.12	0.22	0.14	0.12	—
	20	0.4	0.20	0.19	0.20	0.20	0.19	0.19	−5
	50	1	0.12	0.23	0.24	0.11	0.40	0.40	+70
	100	2	0.15	0.25	0.24	0.16	0.18	0.16	−25

Synaptosomes were incubated and processed as described in Methods. Eight experiments with two synaptosomal preparations are represented.

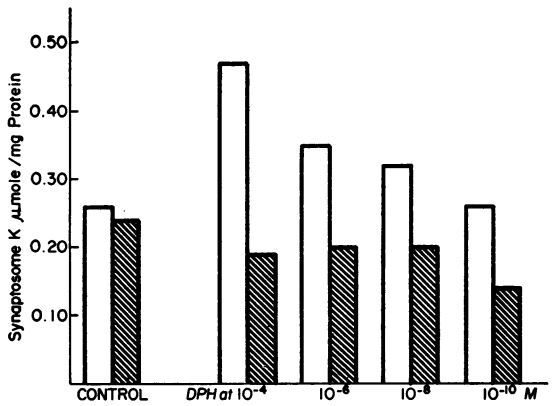

FIGURE 6 In vitro DPH concentrations and its stimulation of K uptake at 50 mM Na and 0.2 mM K. □ control and DPH treated synaptosomes were incubated with (striped bars) ouabain (10^{-4} M). Varying concentrations of DPH were varied as indicated above. The height of the blocks represent K content at 16 min incubations. The above results were obtained from one single synaptosome preparation. Similar results were observed in four other synaptosomal preparations.

accumulation within synaptosomes (Fig. 6). Thus, the stimulatory effects of DPH are apparently mediated to a greater extent through its action on the ouabain inhibitable segment of K accumulation. Furthermore, it appears that under these ionic conditions, the ouabain inhibitable segment of K uptake is the first and most sensitive fraction affected by low concentrations of DPH.

In vivo DPH. The endogenous or base line potassium content of synaptosomes was not significantly changed by in vivo DPH (Table III).

The effects on K accumulation within synaptosomes noted with DPH in vitro were confirmed employing in vivo DPH. The in vitro accumulation of K within synaptosomes was stimulated by in vivo DPH with 50 mM Na and 0.2 mM K in the external medium. The increase in total K uptake roughly corresponded to the ouabain inhibitable segment. Significant stimulation of both total K uptake and its ouabain sensitive segment was observed only after two daily intraperitoneal injections (Fig. 7A). At the 2nd, 3rd, and 5th days of intraperitoneal DPH, ouabain inhibition increased approximately 60% while stimulation of total K accumulation varied at 85% (2 days) to 97% (3 and 5 days).

With 50 mM Na and 10 mM K in the external medium, minimal or no effects on total K uptake were observed and ouabain inhibition in vitro disappeared or was markedly diminished with in vivo DPH (Fig. 7B).

DISCUSSION

The antiepileptic action of diphenylhydantoin has been explained by two different theories: (*a*) that DPH stimulates the active transport of sodium and potassium either directly or indirectly; or (*b*) that DPH specifically blocks the passive translocation of sodium. Evidence in favor of the first theory was provided by experiments of Woodbury (1). Utilizing whole animal brains subjected to maximal electroshock seizures, he demonstrated that DPH increased Na^{22} turnover and decreased the electroshock (EST)-induced elevations of intracellular sodium. These results were interpreted to signify that DPH stimulated the active transport of sodium under conditions of depolarization induced by electroshock. In these experiments, no alterations were noted in potassium although a minor decrease in intracellular potassium content or a shift of K from neuronal to glial compartments would not have been detected by the techniques employed. In contrast to Woodbury's theory, several observations have been interpreted to support a primary blocking action of diphenylhydantoin on the downhill or passive movement of sodium ions (6–8). Experiments on lobster nerves demonstrated that DPH decreased intracellular levels of sodium with no effect on intracellular potassium; the fall in sodium content induced by DPH occurred in the presence of ouabain. These results led to the interpretation that DPH blocked the passive influx of sodium since active sodium pump mechanisms had been inactivated by ouabain.

The critical question is which of the two hypotheses best explains our results with potassium transport in synaptosomes. Our observations clearly demonstrate diphenylhydantoin enhancement of potassium uptake under two conditions: (*a*) with 50 mM sodium and less than 2 mM potassium in the absence of ouabain, and (*b*)

TABLE III
The Effects of In Vivo DPH on Base Line K Content of Synaptosomes

No. of Daily Intraperitoneal DPH injections	Experiment	Control	DPH	Stimulation (+) or inhibition (−)
		μmoles K/mg protein		%
1	1	0.12(±0.009)	0.14(±0.009)	+9.8
	2	0.15(±0.007)	0.14(±0.007)	−6
	3	0.22(±0.005)	0.22(±0.004)	—
2	1	0.09(±0.01)	0.09(±0.01)	—
	2	0.17(±0.006)	0.05(±0.005)	−12
	3	0.08(±0.012)	0.07(±0.009)	−6.6
3	1	0.19(±0.004)	0.18(±0.005)	−5
5	1	0.27(±0.002)	0.27(±0.004)	—

Synaptosomes were processed as described in Methods. The values represent arithmetical means and standard deviations from three determinations of intrasynaptic K content. DPH was administered in single doses daily for the number of days designed above.

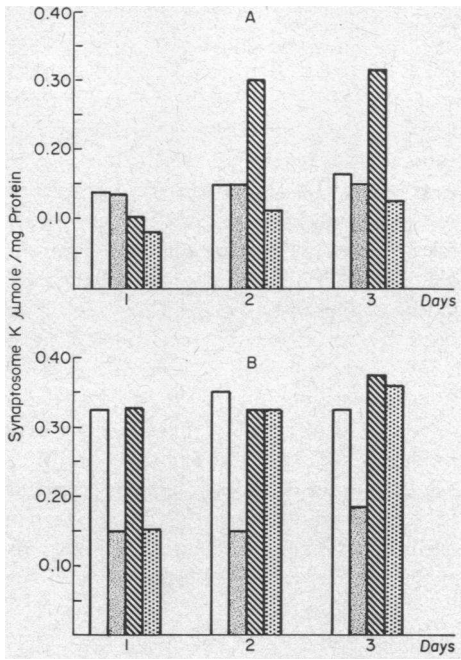

FIGURE 7 Enhancement of in vitro K transport after in vivo DPH. Synaptosomes were incubated and processed as described in Methods. In Panel A, incubation with 50 mM Na and 0.2 mM K is performed. In Panel B, 50 mM Na and 10 mM K is present in the reaction mixture. □ represent control synaptosomes (finely stippled bars) synaptosomes with ouabain (10^{-4} M), (striped bars) synaptosomes from rats treated with intraperitoneal DPH and (heavily stippled bars) synaptosomes from rats which received i.p. DPH, incubated with ouabain in vitro at 10^{-4} M. DPH was administered in anticonvulsant dosages once a day for the number of days indicated above. The values represented by the entire blocks are 16 min incubations. Panels A and B represent six separate experiments from six synaptosomal preparations. Repeat experiments performed in 12 other synaptosome preparations showed similar effects of in vivo DPH.

with 50 mM sodium and 10 mM potassium in the presence of ouabain. These results were obtained when DPH was administered to the animals in vivo or incubated with synaptosomes in vitro. With optimal or high sodium (50–100 mM) and low potassium (less than 2 mM) concentrations synaptosomes demonstrated a gradual loss of endogenous potassium content. A similar state was observed with 50 mM sodium and 10 mM potassium in the presence of ouabain. Under these conditions, DPH not only prevented potassium efflux, but also stimulated potassium accumulation to a level greater than the initial value.

It is difficult to explain these data by assuming that diphenylhydantoin simply blocked potassium efflux. Hypothetically, a block to potassium efflux should result in the maintenance of existing levels of potassium with no increase in synaptosome potassium content above initial values. Yet DPH consistently stimulated synaptosome K levels to higher than baseline values. The fact that ouabain could reverse the DPH induced stimulation of K uptake at high Na and low K concentrations offers evidence that synaptosomal sodium-potassium-ATPase may be involved. These results are in agreement with previous observations that synaptosomal and microsomal sodium-potassium-ATPase is stimulated by DPH under conditions of high sodium and low potassium concentrations (9–11).

However, the action of DPH on the active transport of K must be reconciled with the observation that at 50 mM Na and 10 mM K, DPH enhanced potassium uptake in the presence of ouabain. The latter data might be explained by stimulation of K influx which is not dependent on sodium-potassium-ATPase. Such a transport system for K could conceivably be uphill and independent of the "Na pump." In red blood cells, a second sodium pump has been used to explain sodium efflux in the presence of external sodium although this notion has recently been questioned (12, 13). In synaptosome fractions, the true extent to which K influx is insensitive to ouabain can only be defined by relating the ion movements to intracellular space volumes and further work is needed in this regard. Another way in which the present results might be interpreted and one which we favor is to consider that sodium and potassium concentrations are critical in determining the stimulatory effects of DPH and that DPH and ouabain may have similar sites of action in the synapse membrane. According to such an interpretation, the in vitro stimulation by DPH with 50 mM Na, 10 mM K and ouabain results from its reversal of ouabain action; in the absence of ouabain, DPH has no significant effects at 50 mM Na and 10 mM since active transport is already optimal. This interpretation is supported by our data which showed that: (*a*) at 50 mM Na and 10 mM K, the effects of ouabain on K transport are not observed within synaptosomes pretreated in vivo with DPH; (*b*) DPH induced stimulation of K uptake under high Na and low K concentrations is reversed by ouabain. Previous experiments on DPH which demonstrated stimulation of Na-K ATPase activity at high sodium-low potassium concentrations and which equally emphasized the absence of any significant effects by DPH at optimum ionic conditions, lend further support to this interpretation. These results suggest that DPH may lower the K_m of potassium for the sodium-potassium-ATPase (3).

Our experiments therefore are compatible with Woodbury's hypothesis that DPH stimulates the active transport process. However, in our system this is evident only under those conditions where potassium is leaking out of the synaptosomes, a condition analogous to the depolarized state. DPH had inhibitory or no effects on

potassium uptake within synaptosomes under other ionic conditions. Our observations do not distinguish between direct and indirect effects of DPH on the membrane bound Na-ATPase. However, the data suggest that the effects may be indirect and may depend upon the specific membrane structure, the conformation of the depolarized state and the ionic environment.

It is possible to reconcile the effects of DPH on peripheral nerves, and on synaptosomes by assuming differences in membrane structure. Some pharmacologic agents are known to affect different membranes in different ways. Tetrodotoxin blocks passive sodium conductance in peripheral nerves while it has no demonstrable effects on synapses of the stellate ganglion of the squid (14), and on frog spinal cord synapses (15). Pentylenetetrazol, a known depolarizing agent of central synapses has no effect on intracellular sodium and potassium contents of lobster nerves (7). DPH presently considered to have no effects on ion transport in red blood cells (6) may exert its major effects on passive sodium conductance in peripheral nerves while its effect on synapse membranes may depend upon its interaction with specific membrane structures including the sodium-potassium-ATPase as well as upon specific ionic conditions.

During the first 24 hr, DPH injected in vivo at regular anticonvulsant dosages, for rats had no effects on synaptosomal potassium transport in vitro. This observation is consistent with clinical studies which demonstrate that DPH at the usual clinical dosages required several days to achieve satisfactory anticonvulsant effects (16). Experimental studies show that DPH rapidly enters the cerebral cortex within minutes after intracarotid injection (17). In the cat cerebral cortex, the concentration of DPH 2 min after intracarotid injection is as high as that achieved at 30 min while at 24 hr it is only slightly lower than levels achieved at 3 hr. Similar rates of entry have recently been observed in rat cerebral cortex after intraperitoneal injection (18). The observed delay in the action of diphenylhydantoin on both synaptosomal potassium transport in vitro and clinical seizures could have similar explanations. The critical element could be that diphenylhydantoin may have to reach the synapse terminal before it can exert its effects. If diphenylhydantoin must be transported from a proximal site to the synaptic terminal, such a time lag for effectiveness may be related to the time for its appearance at the synapse.

Additional work is necessary to determine the relevance of these in vitro observations for understanding how diphenylhydantoin modifies convulsive activity in vivo. It is reasonable to suspect synaptic terminals as the sites of action for DPH in its control of seizures since the electrophysiologic expression of epilepsy consists of high amplitude excitatory postsynaptic potentials and dendritically originating action potentials (19). However, changes in intracellularly recorded synaptic events induced by in vivo DPH comparable with those described above have yet to be shown. Supportive evidence for DPH effects on presynaptic events might lie in the demonstration of secondary stimulation or inhibition of neurohormone release from the synaptic membranes under varying ionic conditions in the presence of DPH. Recently, such evidence has been provided by studies of pancreatic tissues and isolated islets of Langerhans where DPH modified insulin secretion through effects on cations and sodium-potassium-ATPase (20). Such studies are in accord with the hypothesis that DPH may act by stimulating the active transport of sodium and potassium and thereby reduce the output of transmitter associated with the convulsive state.

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