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RELATIVE EFFECTS OF HYPERBARIC OXYGEN ON CATIONS AND CATECHOLAMINE METABOLISM IN RATS: PROTECTION BY LITHIUM AGAINST SEIZURES

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SUMMARY

Analysis of lithium (Li^+) in the brain and blood after intraperitoneal injection (i.p.) shows that initially its concentration is high in blood and negligible in the brain. Subsequently its concentration increases in the brain and disappears from the blood. Lithium itself affects neurological actions but the mechanisms remain obscure. It also modifies the toxic action of oxygen at high pressure (OHP), which causes convulsions, either suppressing or exacerbating it. These clearly separate effects correspond with the presence of Li^+ in the blood (suppression) or in the brain (exacerbating). Determination of the effect of Li^+ and OHP upon cations, catecholamines, ammonia, tyrosine hydroxylase, and monoamine oxidase in brain and blood tissue showed that there was very little correspondence between changes in the cations either with Li^+ or the toxic effects of OHP. On the other hand, OHP developed a sustained blood and brain hyperammonemia in rats which could be negatively modified by Li^+ in the blood. The latter effect also corresponded with a prolongation of convulsive latency. Changes in brain catecholamines, tyrosine hydroxylase, monoamine oxidase and tyrosine were effected by Li^+ and potentiated by OHP. These data suggest that Li^+ and OHP mediate their effects relatively more through developing hyperammoneic states in both blood and brain than by altering cation concentrations in these tissues.

Abbreviations: COMT, catecholamine O-methyl transferase; MAO, monoamine oxide; OHP, oxygen at high pressure.

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INTRODUCTION

Lithium pretreatment in rats before high pressure oxygen (OHP) exposure has been reported to delay the onset of convulsions [1]. Lithium seemingly exerts a protective action against convulsions produced by oxygen toxicity only when its concentration is high in the blood shortly after its injection i.p. as a lithium salt. Several hours after i.p. injection lithium is concentrated in brain tissue and at this time potentiates the convulsive state produced by OHP [2]. It has been proposed that the protective effects of lithium against convulsion may be due to its preventing hyperammonemia, a condition which is known to exert toxic effects [2]. The mechanism by which lithium augments convulsions in rats is not known however, although lithium is known to enhance deamination of the catecholamines and indoleamines in the brain producing endogenous ammonia [3—8]. It has been suggested that lithium also competes with such cations as Na^+ and Mg^{2+}, in biological systems, at sites which carry them [1,9]. The potential for such cation-cation antagonism modifying membrane conductance and neurological function is obvious. The relative importance of the above two effects of lithium in modifying the toxic effects of oxygen is not known however.

The present study has investigated the correspondence between OHP-induced convulsions and brain and blood ammonia, catecholamines, monoamine oxidase (MAO), tyrosine hydroxylase and cations during lithium treatment.

MATERIAL AND METHODS

Male Sprague--Dawley rats weighing 200--250 g (Canadian Breeding Laboratories, Ontario) were exposed singly to high pressure oxygen for set periods of time or until convulsions occurred. Some groups were injected intraperitoneally (i.p.) with lithium chloride at different times prior to their oxygenation. The various combinations of lithium injections and oxygen exposure investigated are shown in Table I.

Chamber operation

A small chamber of about 100 l was used for hyperbaric oxygenation of animals. All animals were exposed singly for the stated time and experimental condition. Oxygen was flushed through the pressure chamber after the animals were placed in it at a high rate for 10 min. It was then pressurized up to 72.5 psig (lb/in^2 gauge). This pressurization was completed over a period of 6 min and thereafter gas flow was maintained at 5 l/min. The oxygen concentration was measured in the effluent gas with an Applied Electrochemistry 02 Analyser and carbon dioxide with a Beckman LB analyser. Oxygen concentration throughout the exposure was 99 ± 1% and CO_2 was not measurable. At specific time intervals or after convulsions, the rats were decomposed with stops of 4 min at 40 psig, 1 min at

TABLE I

TREATMENT GROUPS, SHOWING COMBINATIONS OF PRIOR LITHIUM CHLORIDE INJECTION i.p. AND HIGH PRESSURE OXYGEN EXPOSURES (OHP) EXPERIENCED BY VARIOUS GROUPS OF RATS

Group number	Group name	Lithium injection (10 mM/kg)	When sacrificed	Time of OHP exposure after Li^+ injection	Duration of OHP exposure
1	Control N Normal	not injected	as control	not injected	not exposed
2	02-NC normal convulsed	not injected	after OHP convulsion	not injected	until convulsion
3	Li-¼-control	yes	15 min post injection	not exposed	not exposed
4	Li-¼ O_2-C	yes	after OHP convulsion	0.25 h post injection	until convulsion
5	Li-¼ O_2-NC	yes	after OHP without convulsion	0.25 h post injection	same as group 2 (45 min)
6	Li-24 control	yes	24 h post injection	not exposed	not exposed
7	Li-24 O_2-C	yes	after OHP convulsion	24 h post injection	until convulsion
8	Li-48 control	yes	48 h post injection	not exposed	not exposed
9	Li-48 O_2-C	yes	after OHP convulsion	48 h post injection	until convulsion
10	Li-1W control	yes	1 week post injection	not exposed	not exposed
11	Li-1W O_2-C	yes	Li^+ and OHP after convulsion	1 week post injection	until convulsion

30 psig, followed by 3 min continuous, slow decompression to ambient pressure [10]. Under these experimental conditions, 10 min elapsed between the beginning of decompression and collection of blood and brain tissue.

Collection of blood and brain samples

Blood and brain samples were taken and analyzed as described previously [2,11]. Briefly the abdomen of each animal was opened under light ether anesthesia and blood was taken from the bifurcation of the abdominal aorta. The animal was decapitated and the brain was removed intact.

Blood samples were centrifuged and the serum separated. Part of the serum was processed immediately for estimation of ammonia and amino acids and the remainder was frozen with glutathione at −20°C for later estimation of catecholamines.

Brain samples were frozen rapidly in liquid nitrogen. These samples were later analyzed for ammonia, MAO, catecholamines, tyrosine hydroxylase and cations. Since in this experiment our interest was in the collection of blood as well as brain tissue, a liquid nitrogen freezing technique was not used. The differences in concentration of some brain metabolites determined using either the freezing or exsanguination techniques were not significant (Table II).

Biochemical analysis

Blood catecholamines: Serum samples were thawed and centrifuged to free the sample of particulate matter. Clear samples were incubated with *S*-adenosyl-L-C^3H_3 methionine and COMT for 1 h as described by Passon and Peuler [12]. After incubation, the tubes were returned to an ice bath and the reaction was stopped by adding borate buffer at pH 11.00. The metabolites of epinephrine and norepinephrine, metanephrine, and normetanephrine, respectively, were extracted in a mixture of toluene and isoamyl alcohol (3 : 2 v/v). The organic phase was separated and transferred to a 13-ml centrifuge tube containing 0.1 ml of 0.1 M acetic acid. The metabolites were extracted back in the acetic acid phase. Tubes were centrifuged and frozen in a dry ice/acetone mixture. The upper organic phase

TABLE II

SHOWING SOME BRAIN METABOLITE CONCENTRATIONS DETERMINED AFTER SACRIFICING RATS (n = 5) BY RAPID FREEZING AND BY EXSANGUINATION TECHNIQUES

Killing method	Ammonia ($\mu g \cdot g^{-1}$)	Glutamate ($\mu mole \cdot g^{-1}$)	Glutamine ($\mu mole \cdot g^{-1}$)
Rapid freezing	5.10 ± 0.13	8.93 · 0.71	1.03 · 0.49
Exsanguination	5.52 ± 0.27	9.12 · 0.63	0.97 · 0.16

was aspirated and discarded. The aqueous acetic acid phase was thawed and mixed with 1.0 ml of toluene/isoamyl alcohol (3 : 2 v/v), centrifuged and the organic phase was aspirated and discarded as above. 0.15 ml of absolute ethanol was added to the thawed acetic acid. This mixture was streaked on a 2″ × 8″ silica gel plate (250 μm thick GF 254) in a narrow band. Plates were dried and developed in a solvent containing tertiary amyl alcohol/benzene/methyl amine (6 : 2 : 3 v/v/v). The solvent was permitted to migrate from the top of the plate. Plates were removed from the tank and dried thoroughly. Zones were visualized under 254 nm UV light. The top zone contained metanephrine and the bottom zone normetanephrine. Each zone was transferred into a 13-ml tube. The metanephrine and normetanephrine were first extracted in ammonium hydroxide and converted into vanilline as described by Passon and Peuler [12]. The radioactivity was determined in the vanilline fraction.

Brain catecholamines: Brain samples were homogenized with cold 0.2 N perchloric acid (1 : 4 v/w) and centrifuged. The pH of the supernatant was adjusted to 7.5 and 0.1 ml was used for estimating adrenaline and noradrenaline as described above for blood catecholamines.

Brain tyrosine hydroxylase: Brain samples were homogenized with 0.25 M sucrose (1 : 15 parts w/v) at 0°C. 0.5 ml of homogenate was incubated with 0.2 ml, sodium acetate buffer (1.0 M, pH 6.0); 0.1 ml, $FeSO_4$ (10 mM); water (to 1.0 ml total vol.) and 2 amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine (DM pH 4, 10 mM in 1 M mercaptoethanol). The mixture was preincubated for 3 min at 30°C and 100 μl of L-[3,5-^{3}H]tyrosine was added. This mixture was then incubated further at 30°C in air for 15 min. After incubation, 0.1 ml of 25% TCA was added. Ten minutes later, the mixture was centrifuged and the supernatant transferred to a Dower-50 M^+ column (0.5 × 2 cm). The protein precipitate was washed with 1.0 ml of distilled water added through the column. The combined effluent was collected in a counting vial containing 15 ml toluene and liquiflor. Radioactivity was counted and enzyme activity was calculated as described by Levitt et al. [13].

Brain monoamine oxidase: The homogenate for tyrosine hydroxylase estimation was centrifuged at 100 *g* for 10 min at 0°C. One millilitre of clear supernatant was used for analysis of monoamine oxidase. Details of the analytical procedure are described by Weissbach et al. [14].

Brain and serum cations: Serum and brain cations were estimated by the methods of Amdisen [15] using flame photometry.

Serum ammonia: Serum ammonia was analyzed by using the Sigma kit (No. 170-UV). The procedure is based on the enzymatic conversion of L-keto glutarate to glutamate in the presence of ammonia. 0.2 ml of serum was mixed with 3.0 ml of ammonia assay reagent. After 5 min the initial absorbance was recorded. In each sample, 0.02 ml of L-glutamate dehydrogenase was added. The final absorbance was recorded after 5 min and ammonia concentration was calculated from changes in absorbance.

Brain ammonia: Brain tissue was homogenized in 0.25 M Sucrose (1 : 5

w/v) at 0°C. The homogenate was centrifuged at 1000 *g* for 10 min. One millilitre of clear homogenate was treated with 0.2 ml sodium tungstate (10%) and 0.2 ml sulfuric acid (2 N). After mixing, the samples were centrifuged at 3000 rev./min for 10 min. Each 0.8 ml of the supernatant was transferred to a 20-ml test tube and neutralized with 0.2 ml of 1 N NAOH. Each sample together with a reagent blank and standard was mixed with 1.0 ml phenol reagent, 7.0 ml phosphate buffer (pH 9.8), 0.5 ml nitroprusside and 0.5 ml antiformin. The mixture was incubated at 37°C for 10 min. The blue color of indophenol formed with ammonia was measured at 660 nm taking the blank as reference [16].

STATISTICS

A one-way analysis of variance was used to determine homogenity of variance between and within groups. A student-Newman-Keuls Multiple range test was used to determine significant differences between homogeneous subsets, within which, the highest and lowest means differed by not more than the shortest significant range for a subset of that size.

RESULTS

The brain and blood showed 2 distinct profiles of Li^+ during the period following i.p. injection of lithium chloride in rats. Initially blood Li^+ was high but it was not measurable in brain tissue (Fig. 1).

Later (24 h onwards as measured here) Li^+ was significantly increased in the brain and had almost disappeared from the blood. This profile obviously had effect upon the development of convulsions due to exposure of the animals to OHP. Convulsions were delayed when Li^+ was present in blood but precipitate when Li^+ was concentrated in the brain (Fig. 1).

Changes in the brain cation content with Li^+ concentration and the accompanying change in susceptibility to OHP induced convulsions were indefinite. No differences in potassium or magnesium concentration between control animals and those in any experimental group were apparent either before or after OHP induced convulsions. A low brain sodium level was determined in only one experimental group prior to OHP exposure (Li-48) and no differences between convulsed animals and their preconvulsive controls was determined in any group.

No significant changes in potassium or magnesium in the blood occurred in response to Li^+ injection or to OHP exposure in any group. Sodium concentration in Li-15 rats was significantly low compared to normal ($P < 0.05$). Following convulsions produced by OHP exposure sodium increased significantly compared to the preconvulsive control value in all groups ($P < 0.05$). In the group of animals drugged with lithium chloride prior to oxygen exposure for a time equal to the normal undrugged convulsion latency (Li-¼ O_2NC) the sodium concentration was intermediate between the pre-convulsive control value and the Li-¼ O_2-C value.

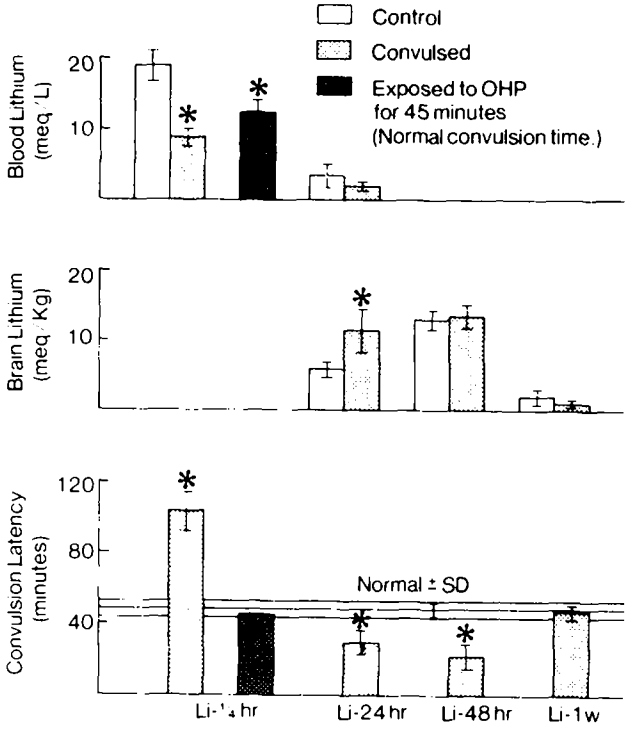

Fig. 1. Blood and brain lithium concentrations and convulsion latencies (mean ± S.D.) in groups (n = 5) of normal (N) and lithium injected rats prior to and after convulsions induced by high pressure oxygen (OHP). Li^+ injected animals were exposed to OHP after injection at the various times indicated. *Significant ($P < 0.05$) when compared with corresponding control.

Li^+ caused no change in brain adrenaline concentration except in Li-24 rats prior to OHP exposure. Adrenaline decreased significantly in all groups after convulsion compared to control values ($P < 0.05$).

Noradrenaline was significantly low in the brain prior to OHP in only one group (Li-48) ($P < 0.05$). A change in noradrenaline was apparent after OHP induced convulsions in only one group. In Li-¼ O_2NC rats exposed to OHP for a time which normally produced convulsions in unprotected animals the noradrenaline concentration was significantly low compared to the control state ($P < 0.05$) (Fig. 2).

Brain tyrosine hydroxylase activity was increased significantly ($P < 0.05$) in Li-24 and Li-48 groups before oxygen exposure and was not different from normal, undrugged animals in the other groups. Its concentration in all groups was significantly increased ($P < 0.05$) after OHP induced convulsions. MAO was similarly elevated in Li-24 and Li-48 groups ($P < 0.05$) prior to OHP and significantly elevated post convulsion in all groups ($P < 0.05$). Tyrosine, precursor of noradrenaline in the brain, was significantly low in Li-48 animals compared to normal. After OHP convulsions

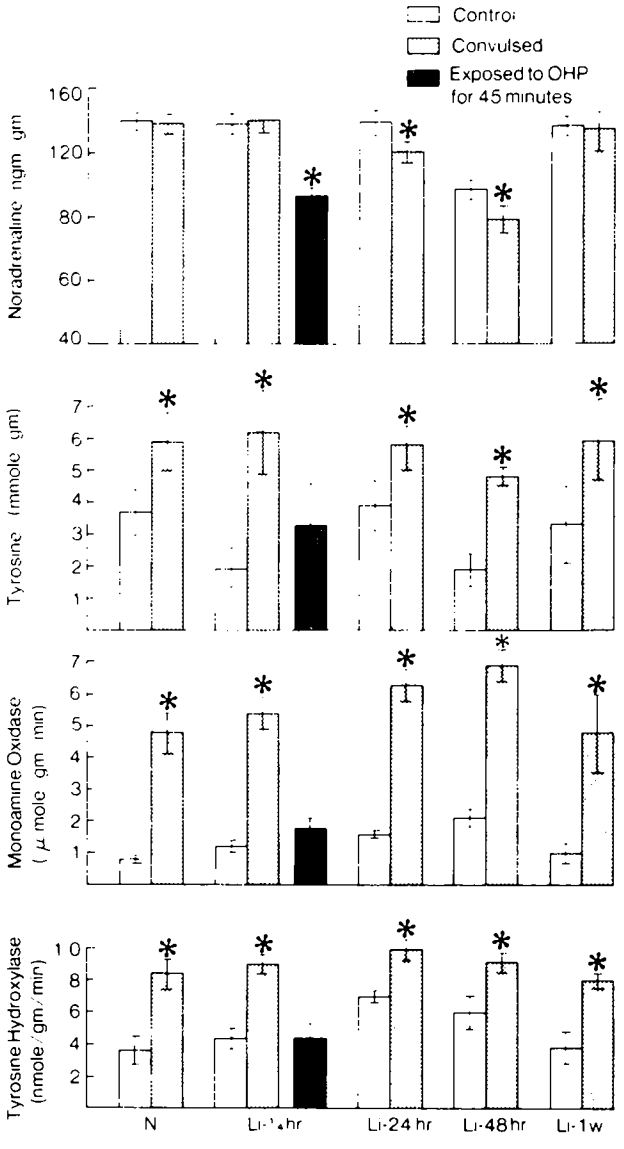

Fig. 2. Brain noradrenaline and tyrosine concentrations and activities of monoamine oxidase and tyrosine hydroxylase respectively (mean ± S.D.) in normal (N) and lithium treated groups (n = 5) exposed to OHP at various times after injection as indicated. *Significant ($P < 0.05$) when compared with corresponding control.

tyrosine was significantly elevated in all groups ($P < 0.05$). Neither MAO nor tyrosine hydroxylase activity was elevated in rats protected with Li^+ and exposed to OHP for a period equal to the convulsion time of normal unprotected animals (Fig. 2).

Blood adrenaline was significantly elevated ($P < 0.05$) in Li-24 and Li-48

rats and unchanged from normal in the other groups prior to OHP. Convulsions produced significant elevations of adrenaline in all groups except in Li-¼ O_2NC animals (Fig. 3).

Similarly nonadrenaline concentrations were significantly elevated ($P < 0.05$) prior to OHP in Li-24 and Li-48 animals and significant elevations of noradrenaline accompanied convulsive activity in all groups ($P < 0.05$) except Li-¼ O_2NC animals (Fig. 3).

Ammonia was normally present in definite amounts in rat brain (Fig. 4). Hyperammonemia accompanied high pressure oxygen exposure in all groups and the finally attained concentrations after convulsion were also similar. Li^+ protected animals exposed to OHP for a time equal to that normally causing convulsion in unprotected animals (Li-¼ O_2NC) had significantly less brain ammonia than any other group ($P < 0.05$) concomitantly convulsion latency was significantly increased ($P < 0.05$).

Hyperammonemia due to OHP exposure was suppressed in the blood by the presence of Li^+ if oxygen exposure occurred soon after i.p. injection of lithium chloride (Li-¼ O_2NC animals) (Fig. 4). Where the hyperammonemia associated with OHP had been suppressed following its i.p. injection, lithium in the blood was concomitantly significantly depleted (Fig. 1).

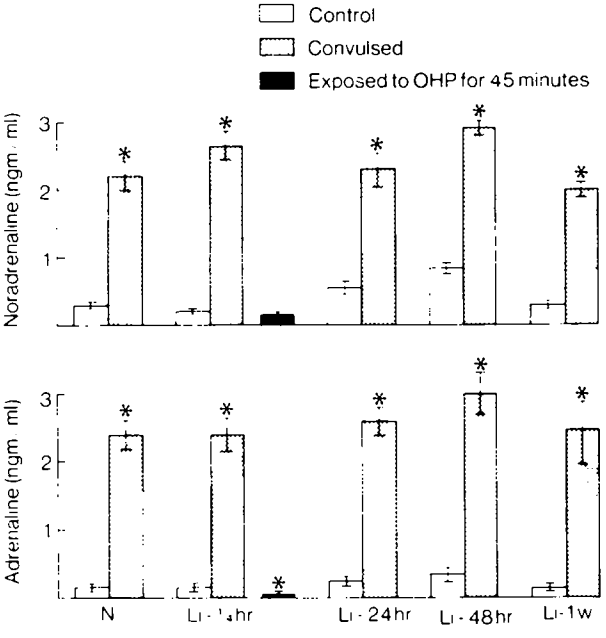

Fig. 3. Blood noradrenaline and adrenaline (mean ± S.D.) in normal (N) and Li^+ injected groups of rats ($n = 5$) exposed to OHP at various times after injection as indicated. *Significant ($P < 0.05$) when compared with corresponding control.

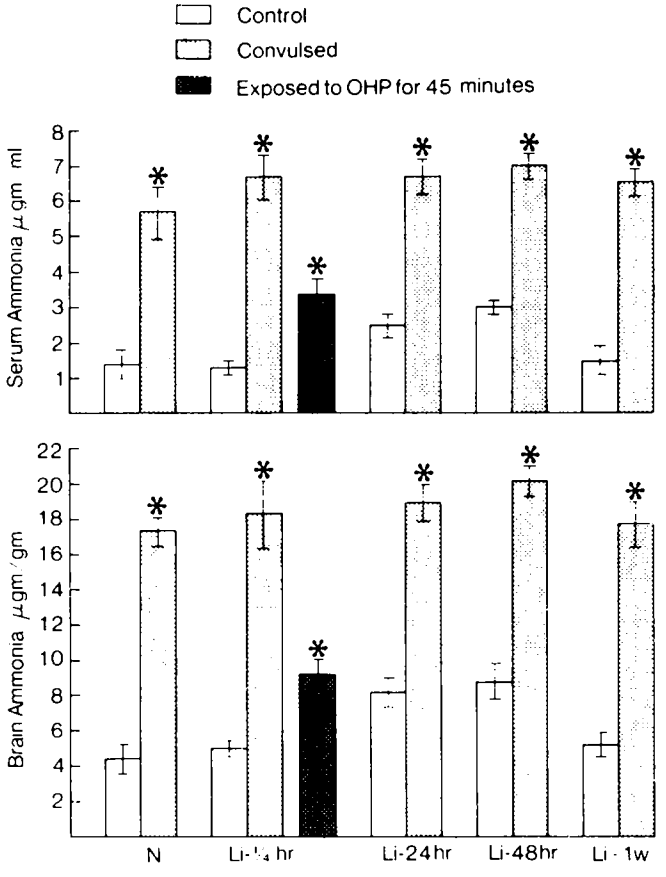

Fig. 4. Serum and brain ammonia concentrations (mean ± S.D.) in normal (N) and lithium injected groups of rats (n = 5) prior to and after OHP induced convulsions. Li^+ injected animals were exposed to OHP after injection at the various times indicated. *Significant ($P < 0.05$) when compared with corresponding control.

DISCUSSION

The present study confirms previous work [1,2] that the concentration of lithium after i.p. injection is first very high in blood and only later is it present in high concentrations in the brain. Lithium seems depleted in the blood in proportion to the degree in which hyperammonemia is suppressed during OHP and both seem related to the increased convulsion latency observed in Li-¼ animals.

Figure 1 shows that Li^+ is protective against OHP when its concentration is high in the blood but that it potentiates convulsions when its brain concentration is high.

The dual role of Li^+ either inducing or inhibiting convulsive action during OHP has been variously ascribed to its competitive replacement of Group I and Group II cations either in the CNS or peripheral neural cells [1], to its

effect upon both CNS and circulating monoamines [3,4], and to its effect as a buffer against a developing hyperammonemia which depletes energy sources and some stabilizing neurotransmitters in the brain [2,17].

Effect of Li^+ on cations in the brain and blood

The dependence of convulsion latency upon the relative blood and brain Li^+ concentrations (Fig. 1) has been attributed by several investigators [1,18] to lithium's displacement of sodium from cells although Pappano and Volle [19] had shown previously that this does not happen in the synapse. Our own results show that far from exerting a protective action against OHP lithium's presence in the brain potentiates the convulsive activity associated with high pressure oxygen exposure. Twenty-four hours after i.p. Li^+ injection brain sodium concentration was unchanged despite a significantly decreased convulsion latency. At this time, both prior to and after convulsion Li^+ levels were significantly increased in all animals. Forty-eight hours post injection when convulsion latency was even shorter, both control and oxygen convulsed animals showed significantly increased brain lithium and decreased brain sodium. These values were also significantly different (Li^+ higher, Na^+ lower) from Li-24 animals. Thus, Li^+ in the brain seems to be important in affecting the convulsive state. Its action on other systems rather than simply upon sodium levels in the cell is also clearly indicated since convulsions always occur with high brain Li^+ but not necessarily with changed Na^+ concentrations. In the blood, the high blood lithium seemed only to affect sodium concentrations. Serum potassium and magnesium were both unaffected by high serum lithium either prior to or post convulsions in any group. However, serum sodium concentration in animals protected by lithium and exposed to oxygen for a time equivalent to the mean time for convulsion of control unprotected animals was intermediate between control levels and those observed when protected animals finally convulsed. The latter group of animals however were able to tolerate OHP more than twice as long as any other group before convulsions occurred.

Displacement of sodium from muscle cells by lithium, which has been observed both to interfere with neuromuscular responses [18] and to decrease miniature m.e.p. plate current and conductance [20], might offer an explanation for a decreased ability of muscle to reflect the toxicity induced in the CNS or peripheral neurons by OHP. The diuretic effect of Li^+ upon Na^+ [9,21] may also be an important modifier of muscle action. However, these would be pseudo-secondary effects, making a real, developing toxicity.

We cannot propose strong arguments from our data which would link the potentiation or inhibition of OHP linduced convulsions exclusively to the action of lithium upon other cations, either in the brain or other tissue.

Effect of Li^+ on catecholamines in brain and blood

Intraperitoneal injection (i.p.) of lithium chloride in normal rats is known

to alter the metabolism of nonepinephrine, dopamine and serotonin in the brain [2–4,6–8]. In the present study there was no apparent immediate effect of i.p. lithium injection upon selected brain monoamines or upon some of the ezyme activities important in their synthesis or degradation. Twenty-four hours later, although the concentrations of adrenaline and noradrenaline in the brain were still unchanged, the activities of tyrosine hydroxylase and the degradative enzyme MAO were both increased relative to undrugged animals, although the ratio tyrosine hydroxylase/monoamine oxidase was relatively unchanged. This latter ratio may be taken as an approximate indicator of the turnover rate of the catecholamine pool in the brain. Conceptually this relative "activity" ratio is useful since it allows one to appreciate that convulsions greatly increase the activity of MAO relative to tyrosine hydroxylase, although the activity of both increased significantly. Lithium's presence in the brain potentiates the activity of MAO in animals prior to any oxygen exposure and this was most evident in Li-48 rats where the relative "activity" ratio was lowest both prior to and after convulsions.

We have shown previously [10] that the time course of brain noradrenaline change in response to OHP is first to decrease and then to increase back to normal levels while at the same time tyrosine hydroxylase activity increases substantially and the precursor substrate, tyrosine, is mobilized.

The degradation of monoamines in both the CNS and periphery whether by MAO or catecholamine *O*-methyl transferase (COMT) is accompanied by deamination and hyperammonemia. Circulating adrenaline augmented from the adrenal glands must suffer the same fate. Ascribing the toxic (i.e. convulsive) effects of OHP to a developing hyperammonemia, susceptible to modification by agents inhibiting or augmenting ammonia production, is both attractive and compelling. Figure 4 shows the developing degree of hyperammonemia as brain Li^+ increases. This profile of hyperammonemia in blood and brain is very similar to that shown in Table III previously published by us [2]. The buffering action of lithium may be judged from the difference between the normal washout Li^+ concentration in the blood and that which actually occurred during OHP as Li^+ slowed the accumulation of ammonia and delayed convulsions.

The results of the present experiment which undertook the simultaneous study of catecholamines, cations, ammonia concentrations and some enzymes activities in the brain and blood of normal and Li^+ protected animals prior to and after convulsions agree well with other experiments which have studied these parameters separately. They are consistent with and provide an integrated explanation of previous literature showing that:

(i) tyrosine hydroxylase is acutely inhibited by OHP but potentiated by Li^+ [7,22];
(ii) an increase in MAO activity accompanies increases in the activity of toxic-free radicals formed during OHP exposures [23];
(iii) adrenalectomy alleviates the convulsions of OHP [24,25],

TABLE III

RAT ARTERIAL BLOOD METABOLITES AFTER OXYGEN BREATHING AT 6 ATA (MEAN ± S.E.), FOR TIME TO CONVULSION (min), AMMONIA (μg/ml), AMINO ACID (μmole/100 ml), UREA (mg/100 ml) AND Li^+ (m-equiv/l) AFTER LiCl INJECTION (i.p.) BEFORE OXYGEN BREATHING

Group	N	O_2-NC	Li-¼ O_2NC	Li-¼ O_2C	Li-24 O_2C
Metabolite					
Li^+ normal washout after injection[a]	—	—	20.08 ± 4.6	18.64 ± 1.94	2.40 ± 0.53
Exposure time	—	50 ± 10	50	115 ± 9	30 ± 6
Li^+ after oxygenation	—	—	9.50 ± 0.39	9.86 ± 0.67	2.26 ± 0.41
Ammonia	2.12 ± 0.30	6.82 ± 0.86	3.64 ± 0.44	8.59 ± 0.54	7.72 ± 0.95
Glutamate	3.50 ± 0.62	2.10 ± 1.42	3.18 ± 0.89	3.44 ± 0.54	3.02 ± 0.33
Glutamine	16.98 ± 0.73	22.66 ± 4.09	19.40 ± 3.07	22.70 ± 2.14	25.62 ± 1.72
Aspartate	1.58 ± 0.38	2.00 ± 0.39	1.84 ± 0.42	1.50 ± 0.34	1.50 ± 0.32
Argenine	1.80 ± 0.29	3.64 ± 0.51	2.50 ± 0.40	4.40 ± 0.46	4.18 ± 0.27
Urea	30.60 ± 4.75	51.92 ± 6.45	30.22 ± 2.25	61.72 ± 2.39	60.02 ± 1.01

[a]Normal washout of lithium from blood at times after injection approximately equal to the time of sacrifice of the various groups (i.e. 1 h, 2 h and 24 h, respectively). (Modified with permission from J. Physiol. (Lond.), Banister et al. 260 (1976) 587—596.)

(iv) lithium in the brain potentiates MAO activity enhancing endogenous production of ammonia in the brain from catecholamine deamination [3,4];
(v) exogenously produced ammonia has access to brain tissue from the CSF by means of an active transport system [26] in addition to a pH dependent non-ionic diffusion gradient from arterial plasma [27];
(vi) lithium when present in blood exerts a buffering action for circulating ammonia [2], thus reducing the exogenous amount available to penetrate the blood/brain barrier,
(vii) blood hyperammonemia enhances brain and CSF tyrosine concentrations [28,29]; and
(viii) TCA cycle intermediates and respiration in some organ mitochondria are inhibited by ammonia (e.g. liver).

The depletion of brain catecholamines, first shown in rats during OHP by blocking tyrosine hydroxylase activity with α-methyl-*p*-tyrosine prior to oxygen exposure [11], was also observed in the present study in Li-48 animals. The reduction in the relative "activity" ratio seemed sufficient to deplete brain catecholamines significantly in Li-48 rats prior to oxygen exposure and to cause a further significant decrease in them after OHP induced convulsions. Other investigators [30] have found that no direct effect upon convulsion latency is obtained by experimentally manipulating the levels of brain catecholamines.

When protection against OHP induced convulsions was provided by high lithium levels in the blood (Li-¼ O_2NC rats) no induction of MAO or tyrosine hydroxylase was observed. Concomitantly, neither blood nor brain monoamines reached the levels usually observed after 45 min of OHP exposure. Tyrosine levels also remained low in the brain and circulating ammonia in both blood and brain was only half the concentrations reached in animals not protected by lithium and convulsed after a similar period of oxygen exposure (48 min).

Hyperammonia is an alternative explanation to cation disturbances in accounting for the disrupted neurological state accompanying convulsion action. The effect of hyperammonemia is particularly critical in the brain since an excess diverts glutamate to buffer it [2,3] and this compromises the action of the γ-glutamyl cycle [31] in the transport of amino acids. The transamination of α-ketoglutamate from the Krebs cycle necessary to replenish depleted glutamate reserves must also have a negative effect upon energy production in the brain and further deplete the integrity of the neuronal cell and γ-amino butyric acid is also reduced when brain glutamate concentration declines [1].

It seems that explanation of the action of Li^+ upon OHP induced convulsions is better modelled upon the consequences of a developing hyperammonemia than upon significant cation changes.

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