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Hypertension and (Na–K) ATPase activity in brain

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There is a growing volume of literature on the association between altered sodium metabolism and states of hypertension. The intracellular sodium content is a balance between influx (passive permeability) and efflux which is primarily achieved by active transport linked to potassium uptake by the sodium potassium pump. (Na–K) ATPase is believed to be an enzymic representation of the ouabain sensitive sodium potassium pump. The studies of Pamnani *et al.* [1] indicate that there is a suppression of vascular smooth muscle sodium potassium pump activity in non-genetic low renin hypertensive rats but that there is increased pump activity in spontaneously hypertensive and hypertensive Dahl's salt-sensitive rats. Several papers report that there is an increased sodium permeability in erythrocytes of human hypertensives and spontaneously hypertensive rats and that this increase in permeability is in part compensated for by an increased sodium pump activity [2–4]. Other authors [5] conclude that essential hypertension is associated with an inherited defect in the sodium potassium co-transport system. Forrester and Alleyne [6] recently reported a decrease in sodium pump activity in the leukocytes of patients with pre-eclampsia hypertension. However, no evidence at present exists of changes in (Na–K) ATPase in brain. Such changes might be expected in view of the relationship between (Na–K) ATPase activity and ionic flux across nerve cell membranes [7]. (Na–K) ATPase of synaptic membranes has been implicated in the regulation of transmitter release [8]. In the light of the observation of changes in the monoamine transmitter content of brain areas associated with cardiovascular control in hypertensive rats, we undertook to study the ATPase activity of brain homogenates prepared from spontaneously hypertensive rats and aged matched controls.

Spontaneously hypertensive rats of the Okamot SHR strain and aged match Kyoto Wistar rats were used in this study. A total of 48 rats between the ages of 6 and 12 weeks were used. The systolic blood pressure of each rat was measured on the day preceding (Na–K) ATPase analysis. The animals were lightly anaesthetised with ether and their blood pressures were measured from the tail artery using a photo-electric transducer. The animals were grouped in pairs according to the rank of the BP measurement. The BPs of the SHRs were obviously higher than control in the 10 and 12-week-old animals. The animals were killed by cervical dislocation. The brain was exposed and rapidly removed to ice-cold imidazole buffer pH 7.4. The cerebellum was removed and the brain stem, including pons and medulla, were dissected free; and area of midbrain, including corpora quadrigemina and hypothalamus, was dissected. The cerebral cortex was removed and the striatum obtained from a transverse section of the remaining brain. All the dissected brain areas were stored in chilled imidazole buffer before being homogenised by six thrusts of a power-driven Teflon pestle. The maximum time of storage in the chilled imidazole buffer was less than 4 min. Studies to investigate the rate of accumulation of fluid during the storage period could account for an increase in sample weight in all samples. There was no significant difference in the % increase in weight between brain areas or between SHRs and controls. The fluid uptake by the samples were 0.12 ± .032 g/g cerebellum; 0.14 ± 0.049 g/g brain stem; 0.16 ± 0.05 g/g midbrain; 0.13 ± 0.02 g/g striatum; and 0.165 ± 0.08 g/g cerebral cortex. Values are means ± S.D. of six experiments. The homogenate was centrifuged at 1000 *g* for ten minutes. The specific activity of the supernatant fraction is some ten-fold greater than in the original homogenate. The supernatant fraction was used in the enzyme assays reported here. This supernatant was designated S_1.

ATPase activity was determined by the rate of release of inorganic phosphate. Membranes were incubated at 37° in 1 ml of imidazole buffer. The membranes were added to a final concentration of approximately 100 μg protein/ml. Total ATPase activity was assayed in a medium containing 135 mM Na, 9 mM K, 4.5 mM Mg, 4.5 mM ATP. The Mg ATPase activity was assayed in 4.5 mM Mg and 4.5 mM ATP. The data presented here was obtained using Boehringer disodium ATP. Rate of release of P_i is linear with respect to time from 15 to 60 min. An incubation period of 30 min was found to be adequate and the ATPase activity was a linear function of membrane protein concentrations in the range of 60–150 μg/ml. The incubations were stopped by adding 4 ml of a solution of 1% ammonium molybdate and 1% lubrol WX in 0.9 M H_2SO_4. The colour was left to develop for 30 min and the OD read on a Pye Unicam SP 6-500. The colour was stable for up to 3 hr. (Na–K) ATPase was estimated from the difference between total ATPase and Mg ATPase. Protein was assayed by the method of Lowry *et al.* [9].

There was no significant difference in the ATPase activity of whole brain homogenates. However, dissection of the brain into five major areas — cerebral cortex, striatum, cerebellum, midbrain including corpora quadrigemina and the hypothalamic areas, and the brain stem including pons and medulla oblongata — revealed a disparity between the ATPase activity of the SHRs and that of the control group at the age of 6 weeks (Table 1). The Mg ATPase activity was highest in the cerebral cortex, 9.3 ± 2.8 μmoles P_i/mg/hr of the control animals, but there was no significant difference between the Mg ATPases of any brain region of controls and those of the SHRs. The specific activity of (Na–K) ATPase was highest in the cerebral cortex of the control animals. However, the (Na–K) ATPase activity of cortical homogenates from SHR was not significantly different from control. The (Na–K) ATPase of the midbrain region from the SHRs was significantly greater ($P < 0.001$) than controls. This result is interesting since Lehr *et al.* [10] have reported gross morphological changes in the mesencephalic region of SHRs. The possibility that this change in enzyme activity preceded the onset of the hypertensive state was suggested by the blood pressure recordings obtained, although it is our opinion that definite conclusions on this matter should rest on the results of more direct blood pressure readings.

Over the following weeks, groups of the animals were sacrificed and the ATPase activities of the midbrain areas and brainstem was studied. There was no significant difference in (Na–K) ATPase activity of brain stem of SHR compared with controls. It is apparent that there is a decrease in the level of (Na–K) ATPase with age. However, this elevation of midbrain (Na–K) ATPase in SHRs was apparent in all groups studies (Table 2).

From the observations of Haeusler *et al.* [11], it seems likely that increased catecholaminergic activity may be prominent in initiating or triggering the hypertension in the developing spontaneously hypertensive rat. Vizzi [8] has recently reviewed the extensive literature on transmitter release and the activity of synaptic membrane (Na–K) ATPase. There is considerable evidence to suggest that factors which stimulate (Na–K) ATPase activity inhibit transmitter release. However, if there was an increase in

Table 1. ATPase activity of S_1 fraction from rat brain homogenates

Sample	Weights (mg)		S_1 Protein concn (mg/ml)		Mg ATPase*		(Na–K) ATPase*	
	Control	SHR	Control	SHR	Control	SHR	Control	SHR
Cerebellum	239.2 ± 12.8	250.7 ± 19.4	1.2 ± 0.1	1.16 ± 0.18	3.25 ± 0.9	3.96 ± 0.8	3.3 ± 1.3	4.1 ± 0.7
BS	139.1 ± 13.6	142 ± 19.7	0.91 ± 0.18	1.12 ± 0.14	3.5 ± 1.2	3.88 ± 1.0	5.18 ± 0.8	4.9 ± 0.57
MB	202.4 ± 19.6	190 ± 14.7	0.97 ± 0.10	0.90 ± 0.1	3.72 ± 0.46	3.85 ± 0.08	4.8 ± 1.5	10.7 ± 2.7†
Striatum	90.4 ± 27.6	105.4 ± 20.1	0.84 ± 0.05	1.05 ± 0.17	3.5 ± 0.51	4.71 ± 0.16	6.1 ± 2.0	7.4 ± 1.5
Cerebral cortex	517 ± 69.3	581 ± 71.1	0.96 ± 0.17	0.85 ± 0.1	9.3 ± 2.8	11.4 ± 1.8	10.9 ± 2.2	11.16 ± 1.45

* Units of μmoles P_i/mg of protein/hr.
† Statistically significant at $P < 0.001$.

Table 2. (Na–K) ATPase of S_1 fractions from homogenates of rat midbrain

Age	SHR			Control		
	Sample weight (mg)	Protein concn (mg/ml)	(Na–K)ATPase (μmoles P_i/mg/hr)	Weight (mg)	Protein concn (mg/ml)	(Na–K)ATPase (μmoles P_i/mg/hr)
6	190 ± 47.0	0.9 ± 0.1	10.7 ± 2.7	202 ± 19.6	0.97 ± 0.1	4.8 ± 1.5†
8	212 ± 37.8	1.0 ± 0.16	5.87 ± 1.85	188.4 ± 26.1	1.04 ± 0.2	2.5 ± 0.15*
10	190.4 ± 17.6	0.96 ± 1.6	3.75 ± 0.35	208.6 ± 36.1	1.2 ± 0.16	2.1 ± 0.4†
12	207.7 ± 20.1	0.93 ± 0.09	4.65 ± 0.51	212.0 ± 31.4	1.08 ± 0.05	1.5 ± 0.1*

The (Na–K)ATPase activity of S_1 fraction from homogenates of midbrain areas from spontaneously hypertensive rats and aged match controls. Results are the means of three experiments ± S.D.
† $P < 0.005$, * $P < 0.001$.

synaptic activity, this is likely to trigger the induction of (Na–K) ATPase [7].

To summarise, there is a significant increase in the (Na–K) ATPase activity in homogenates of midbrain regions from spontaneously hypertensive rats. This increase might reflect changes in the permeability of the membranes to sodium or perhaps an increase in the synaptic activity of that region.

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Evidence that prostaglandin endoperoxides can induce platelet aggregation in the absence of thromboxane A_2 production

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Arachidonic acid causes aggregation of platelet rich plasma (PRP). Platelet cyclooygenase converts arachidonic acid to the prostaglandin endoperoxides PGG_2 and PGH_2 which in turn are converted by platelet thromboxane synthetase to thromboxane A_2 (TXA_2), a very potent aggregating agent [1]. Low concentrations of collagen or thrombin release arachidonic acid from platelet phospholipids. The arachidonic acid is converted via the prostaglandin endoperoxides to TXA_2 and aggregation occurs. The addition of either prostaglandin endoperoxides (PGG_2/PGH_2) or TXA_2 to human PRP causes aggregation but TXA_2 is more potent in this respect [2]. It has been assumed that the aggregatory effects of PGG_2/PGH_2 are due mainly to their conversion to TXA_2 but in describing the effects of substituted imidazoles on platelet aggregation induced by arachidonic acid, Yoshimoto *et al.* [3] noted that 1-heptyl imidazole was less effective as an inhibitor of aggregation than as an inhibitor of thromboxane synthetase. Furthermore, Heptinstall *et al.* [4] observed that selective inhibition of platelet thromboxane synthetase with UK 34787 [2-isopropyl-3(1-imidazolyl methyl) indole] led to inhibition of aggregation by arachidonic acid in PRP from some but not all donors. Needleman *et al.* [5] showed that high concentrations of imidazole completely inhibited the formation of TXA_2 from PGH_2 by human washed platelets without abolishing the aggregatory response and demonstrated that imidazole reduced TXA_2 production in response to collagen, thrombin and arachidonic acid without reducing aggregation. Similarly, Blackwell *et al.* [6] during studies of thromboxane synthetase inhibition by 1-*N*-butyl imidazole observed that platelet aggregation induced by PGH_2 could not be completely overcome even by high concentrations of the inhibitor. A stable chemical analogue of the prostaglandin endoperoxides, U 46619 [(15*S*)-hydroxy, 11α, 9α-(epoxymethano) prosta-5Z, 13E-dienioc acid] has recently been described as having a similar pharmacological profile to TXA_2 on isolated tissues. Whilst this compound is more closely related structurally to the endoperoxides, it acts as an agonist at thromboxane receptors [7]. These observations suggest that endoperoxides and TXA_2 may act at the same receptor and therefore that endoperoxides may have significant aggregatory activity in their own right particularly under conditions where conversion to TXA_2 is prevented.

In order to investigate this proposition we have measured concurrently thromboxane B_2 (TXB_2) production and platelet aggregation in response to collagen in human PRP treated with UK 34787, indomethacin and combinations of the two drugs.

Materials and methods

Materials. UK 34787 was generously provided by Pfizer Ltd. (Sandwich, U.K.). The compound was dissolved in 0.1 N HCl and diluted to working concentrations with 0.154 M NaCl. Collagen was purchased as a 1 mg/ml suspension in buffer from Hormon Chemie (Munich, West Germany). Indomethacin was purchased from the Sigma Chemical Co. (London, U.K.). TXB_2 was purchased from the Upjohn Company (Kalamazoo, MI), and [^{3}H]TXB_2 (100 Ci/mM) was purchased from New England Nuclear (Southampton,