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Noradrenaline stimulation of (Na^+, K^+)ATPase in homogenates of the developing rat brain

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Noradrenaline, dopamine, and 5-hydroxytryptamine have been reported to activate membrane bound (Na^+, K^+)ATPase in various brain preparations [1–8]. Although catecholamine stimulation of (Na^+, K^+)ATPase activity has been well documented, the mechanisms by which catecholamines stimulate the enzyme activity are still poorly understood. Cheng *et al.* [9] have shown that rat skeletal muscle membrane (Na^+, K^+)ATPase activity can be stimulated by various catecholic agents. Stimulation of the enzyme was apparently not specifically a β-adrenergic response since the response of the enzyme to β-adrenergic agonists was less specific in its structural requirements than that of the typical β-adrenergic receptor. Studies on catecholamine stimulation of (Na^+, K^+)ATPase activity in adipose tissue and in various brain preparations have demonstrated that activation is mediated by adrenergic receptors as it can be blocked by α- and/or β-adrenergic receptor antagonists [6, 7, 10–13].

It has been shown recently that the number of β-adrenergic receptors in rat brain is age dependent [14]. The capacity of brain adenylate cyclase to respond to catecholamines, and the concentration of receptors, as demonstrated by ligand binding studies, developed in a parallel [14], suggesting that the development of adrenergic responses in the maturing rat brain is dependent on the appearance of adrenergic receptors. We have now investigated the ontogeny of the response of brain cortical (Na^+, K^+)ATPase to noradrenaline stimulation, and these results are correlated with the reported data on the development of adrenergic receptors.

Pregnant female Wistar rats with known conception dates were obtained from the Canadian Breeding Farm Laboratories in Montreal. Brains were obtained from 16-day post-conception fetuses, and from newborn, day, 14, 21, 28 and 38 post-natal, and adult (60-day) rats. The brains were homogenized in 50 vol. of distilled water (pH 7.5). Some of this homogenate (50 μl) was used for the incubation. (Na^+, K^+)ATPase activity was determined by subtracting Mg^{2+}-ATPase activity (ouabain-insensitive) from total ATPase activity. The medium used for the estimation of total ATPase activity consisted of final concentrations (mM) of: Tris, 115; $MgCl_2$, 5.0; KCl, 6.25; and NaCl, 72.5. Mg^{2+}-ATPase activity was measured in a K^+-free medium consisting of final concentrations (mM) of Tris, 175; $MgCl_2$, 5.0; NaCl, 14; and ouabain, 1.0. In all experiments, the homogenate was preincubated for 10 min at 37° in the presence or in the absence of noradrenaline. The reaction was terminated 10 min after the addition of disodium adenosine triphosphate (ATP) (2 mM vanadate-free ATP, Sigma) by adding 500 μl of ice-cold 12% trichloroacetic acid solution in an ice bath. The content of inorganic phosphate in the supernatant fraction was measured by the method of Fiske and Subbarow [15]. The brain homogenate, and noradrenaline and ATP solutions were made up fresh for each experiment. Our previous reports [6, 7] have shown that the optimum reaction conditions for measuring rat brain cortical (Na^+, K^+)ATPase activity require 5 mM Mg^{2+} and 2 mM ATP (synthetic or vanadate-free), which are similar to the reaction conditions suggested by Skou [16]. The (Na^+, K^+)ATPase activity measured under these reaction conditions is linear for 20 min of the incubation period (not shown), and the inorganic phosphate formed does not exceed 20 per cent of the substrate ATP.

The results in Fig. 1 show that (Na^+, K^+)ATPase activity in brains of 16-day fetal, newborn, and 7-day post-natal rats was low [less than 0.1 μmole Pi(mg wet weight)$^{-1}\cdot h^{-1}$]. The enzyme activity increased gradually after day 7 of post-natal life and then sharply after day 21, reaching a maximum [approximately 1.0 μmole Pi·(mg wet weight)$^{-1}\cdot h^{-1}$] on day 38 and remaining at this level up to day 60 of post-natal life. Fetal Mg^{2+}-dependent ATPase activity was higher than that of (Na^+, K^+)ATPase in 16-day fetus. The Mg^{2+}-ATPase activity increased gradually during the first 7 days of the post-natal period and then increased markedly in activity to 21 days of post-natal development; the activity finally reached a maximum [approximately 2.0 μmoles Pi·(mg wet weight)$^{-1}\cdot h^{-1}$] by day 38 of post-natal life and stayed at the same level up to day 60 after birth.

The response of rat brain (Na^+, K^+)ATPase activity to noradrenaline was measured during the different developmental periods. Enzyme activity was increased by various concentrations of noradrenaline after day 7 of post-natal life. The enzyme activities in brain homogenates of 16-day pre-natal, newborn and 7-day post-natal rats were not

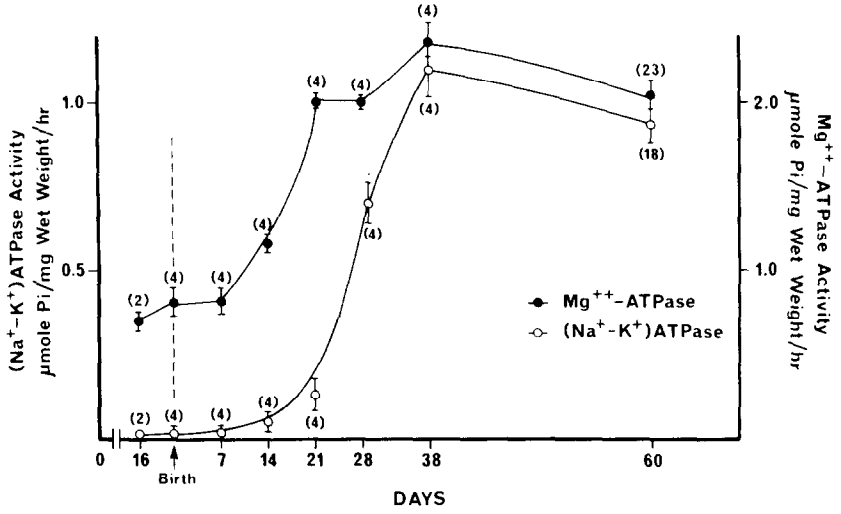

Fig. 1. (Na^+, K^+)ATPase and Mg^{2+}-ATPase in brain homogenates of the developing rat. Fifty microliters (equivalent to 1.0 mg tissue) of rat brain homogenate was preincubated for 10 min at 37°. The enzyme activity was measured as the amount of inorganic phosphate released during 10 min of incubation after the addition of vanadate-free ATP to a final concentration of 2 mM. Each point with an accompanying bar is the mean ± S.E., except for the two points at 16 days, which have S.D. bars. The number of experiments is in parentheses, each experiment was done in triplicate.

stimulated by noradrenaline concentrations ranging from 1×10^{-7} to 1×10^{-3} M (Table 1). At day 14 after birth, (Na^+, K^+)ATPase activity was activated significantly ($0.01 > P > 0.001$) by noradrenaline (1×10^{-6} M). When concentration–response curves of (Na^+, K^+)ATPase to noradrenaline were constructed and the EC_{50} values obtained (Table 1), it was apparent that (Na^+, K^+)ATPase from day 14 post-natal animals was most sensitive to noradrenaline stimulation, with an EC_{50} value of 5.0×10^{-8} M and a maximum stimulation of 800 per cent at a noradrenaline concentration of 1×10^{-4} M. As the rats became more mature, the enzyme became less sensitive to noradrenaline stimulation, as demonstrated by the increase in EC_{50} values which were 5.6×10^{-7} M (21-day rats); 1.2×10^{-5} M (28-day rats); 1.5×10^{-5} M (38-day rats) and 3.3×10^{-5} M (60-day rats).

Studies on the developing rat brain have revealed that (Na^+, K^+)ATPase activity increases during neonatal maturation along with the formation of dendrites and an increase in electrical activity [17–20], suggesting that the increase in (Na^+, K^+)ATPase activity is associated with the development of synaptic activity. Other studies have also shown that there is a correlation between the development of electroencephalographic activity and the appearance of (Na^+, K^+)ATPase activity in rat brain [21]. Similar results were obtained with cerebral cortex of kittens, where an increase in (Na^+, K^+)ATPase activity was noted between 1 and 6 weeks post-partum, which was also the period of rapid rise in spontaneous electrical activity in cortical neurons [22]. We observed that (Na^+, K^+)ATPase activity was low in neonates and that it increased slowly on day 14 and very rapidly from day 14 to day 38 post-partum. The later phase of rapid increase in (Na^+, K^+)ATPase activity seems to correspond to the time period of rapid glial cell proliferation [23]. Mg^{2+}-ATPase activity, however, increased rapidly after day 7 post-partum and reached the adult level by day 28 of post-natal growth. The developmental profile for the Mg^{2+}-ATPase activity appears to correspond with that of the vesicular noradrenaline uptake system [24] and that of synaptogenesis [25].

Of particular interest has been the activation of (Na^+, K^+)ATPase by noradrenaline in the developing rat brain. The enzyme was not sensitive to noradrenaline before day 7 post-partum. During the developmental stage (from 7 to 21 days) that corresponds to the most rapid period of synaptogenesis [25] and the appearance of adrenergic receptors [14], the (Na^+, K^+)ATPase was very sensitive to noradrenaline, as shown by the EC_{50} values of 5.0×10^{-8} and 5.6×10^{-7} M for day 14 and day 21 post-natal brain respectively. During the period when brain (Na^+, K^+)ATPase activity increased rapidly (21–38 days), however, the enzyme was less sensitive to noradrenaline, as indicated by the increase in EC_{50} values for the enzyme stimulation by noradrenaline.

This reduction in the responsiveness of (Na^+, K^+)ATPase to noradrenaline in the maturing brain may be due to the increasing contribution of glial (Na^+, K^+)ATPase to the total enzyme pool. Studies on (Na^+, K^+)ATPase prepared from cultured astrocytes show

Table 1. EC_{50} Values for noradrenaline stimulation of (Na^+, K^+)ATPase activity at the various developmental stages of rat brain*

Rat brain	EC_{50} Value (M)
16-Day pre-natal	†
Birth	†
7-Day post-natal	†
14-Day post-natal	5.0×10^{-8}
21-Day post-natal	5.6×10^{-7}
28-Day post-natal	1.2×10^{-5}
38-Day post-natal	1.5×10^{-5}
60-Day post-natal	3.3×10^{-5}

* EC_{50} Values were obtained from semi-logarithmic plots of concentration-response curves determined from noradrenaline (1×10^{-7} M to ~ 1×10^{-3} M) stimulation of (Na^+, K^+)ATPase of the rat brain homogenate. Results obtained from four to six separate experiments were used to construct the concentration–response curve. In all cases, the S.E. of the experiment was not greater than 11 percent.

† No detectable stimulation of (Na^+, K^+)ATPase by various concentrations of noradrenaline (up to 1×10^{-3} M).

that noradrenaline has only a weakly stimulant action on the enzyme (maximum 35 per cent stimulation with 1×10^{-5} M noradrenaline) (P. H. Wu, L. Hertz and J. W. Phillis, unpublished observations). As the proportion of the enzyme derived from glial cells increases during glial cell proliferation, there will be an apparent reduction in the sensitivity of the total brain enzyme to noradrenaline. This idea is supported by a recent finding reported by Sweadner [26] of two distinct molecular forms of (Na^+, K^+)ATPase in brain; one form may be the neuronal enzyme, and the other a glial cell component.

In conclusion our results suggest a possible relationship between neurogenesis, the development of adrenergic receptors, and (Na^+, K^+)ATPase activation by noradrenaline in the central nervous system of the rat.

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