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Biochemical Pharmacology, Vol. 28, pp. 2847–2849.

0006-2952/79/0915-2847 $02.00/0

Diminished adenylate cyclase responses in frontal cortex and cerebral capillaries of spontaneously hypertensive rats

(*Received* 25 *January* 1979; *accepted* 26 *March* 1979)

A variety of neurohumoral agents, namely beta-adrenergic agonists, dopamine, histamine and prostaglandins, have been shown to stimulate the enzyme, adenylate cyclase, in the frontal cortex and cerebral neurons of laboratory rats [1]. Recently, capillary fractions from the rat cerebral cortex were found to contain a similar adenylate cyclase system sensitive to activation by beta-adrenergic agonists and dopamine [1–3]. In addition, the presence of adrenergic and dopaminergic nerve endings has been demonstrated in cerebral capillaries, and preliminary evidence suggests an involvement of norepinephrine-coupled adenosine cyclic 3′,5′-monophosphate (cyclic AMP) synthesis in the control of capillary permeability, particularly to water [4–9]. Under conditions of chronic hypertension, both blood–brain barrier lesions and cerebral edema development are known to occur, possibly as a result of increased cerebrovascular permeability [10–13]. The latter may indicate a defect in the capillary adenylate cyclase system. An extension of the latter hypothesis may be made to peripheral organs because activation of adenylate cyclase by neurohumoral agents has been shown to be reduced in the myocardium and blood vessels of hypertensive rats [14–17]. In addition, neuronal sites within the frontal cortex have been implicated in the pathogenesis of hypertension [18], and two popular antihypertensive drugs, propranolol (beta-adrenergic antagonist) and clonidine (alpha-adrenergic agonist), when given to spontaneously hypertensive rats (SHR), were found to alter the sensitivity of cortical adenylate cyclase to norepinephrine [19]. Against this background, the present study was designed to evaluate, in the frontal cortex and cerebral capillaries, whether the activation of adenylate cyclase by specified neurohumoral agonists differed between SHR and normotensive rats.

The animals, Wistar Kyoto (WKY) or SHR (obtained from Charles River Breeding Co., LA), were age matched per individual experimental condition (age range was 6.5 to 8 weeks), and the weight ranges were 135–175 g. The rats were decapitated, the brains were rapidly removed, and the cerebral cortices were dissected free and placed in cold (4°) 0.25 M sucrose (100 ml for the capillary isolation) or diglycine buffer (2 mM + 1 mM $MgSO_4$ + 0.2 mM EGTA,* pH 7.4, for the total frontal cortex). For the capillary isolation, the cortices (five per experiment) were freed of pia, chopped into 1 mm sections and filtered through successive pore sizes of nylon bolting cloth (333 and 110 μm, three times each). The suspension was centrifuged at 1500 *g* for 10 min, and the pellet was resuspended in 0.25 M sucrose, filtered (110 μm cloth), and recentrifuged. The pellet was again suspended in 0.25 M sucrose (40 ml), filtered (110 μm cloth) and layered onto a gradient consisting of 1.5, 1.3 and 1.0 M sucrose (5 ml each) and centrifuged at 20,000 rev/min (30 min) using a SW 25.1 rotor. The final pellet containing the capillary fraction, as monitored by phase microscopy [1, 8, 9, 20], was homogenized in cold diglycine buffer and, along with the frontal cortical homogenate, was assayed for adenylate cyclase activity (6 min at 37°) exactly as described previously [1, 2]. Enzyme activity was expressed as pmoles of cyclic AMP formed/mg of protein/min. Enzyme activity in the capillary fractions represented about $\frac{1}{3}$ of the total activity in the cortical homogenate. However, the total homogenate contains structures, e.g. myelin, which possess little enzyme activity. Moreover, other studies show that cellular purification yields progressively to higher adenylate cyclase activities [1]. Student's two-tailed *t*-test was used to compare data between the WKY and SHR, while Student's paired *t*-test was employed to evaluate enzyme stimulation over respective basal activity within a particular strain of rats.

Figure 1 compares the activation of adenylate cyclase in the frontal cortex of the WKY and SHR. Significant ($P < 0.05$) enzyme stimulation (when compared to respective basal activity) was produced in both animal strains by *l*-norepinephrine, dopamine, *d,l*-isoproterenol, histamine (all obtained from the Sigma Chem. Co., St. Louis, MO), salbutamol ($beta_2$-agonist, Allen & Hanbury's Ltd., England), and dobutamine ($beta_1$-agonist, gift from the Eli Lilly Co., Indianapolis, IN). In general, this enzyme activation was evident using agonist concentrations of 1–100 μM; however, in the SHR, dobutamine and salbutamol were not significantly effective at either 1 or 10 μM and histamine was likewise ineffective at 1 μM. In addition, there were significant differences in the activities of adenylate cyclase between the WKY and the SHR. Responses in the SHR were thus lower to norepinephrine (10 μM), dopamine (1–100 μM) and dobutamine (1–100 μM). In general, agonist-induced enzyme activation was lower throughout the SHR; however, the basal enzyme activities were not significantly different between the two strains.

In the cerebral capillary fraction from both strains, adenylate cyclase was elevated significantly by the adrenergic agonists alone (usually 1–100 μM). Histamine was not active, a finding reported previously [1, 2]. In the SHR, significant enzyme stimulation (over respective basal activity) was not evident at the lowest (1 μM) concentrations of norepinephrine, dobutamine or salbutamol. Reduced (significant) adenylate cyclase responses to norepinephrine (1–10 μM), dopamine (1–100 μM), isoproterenol (1–10 μM), salbutamol and dobutamine (both at 1–100 μM) were prominent in the SHR

* EGTA = ethyleneglycolbis(aminoethylether)tetraacetate.

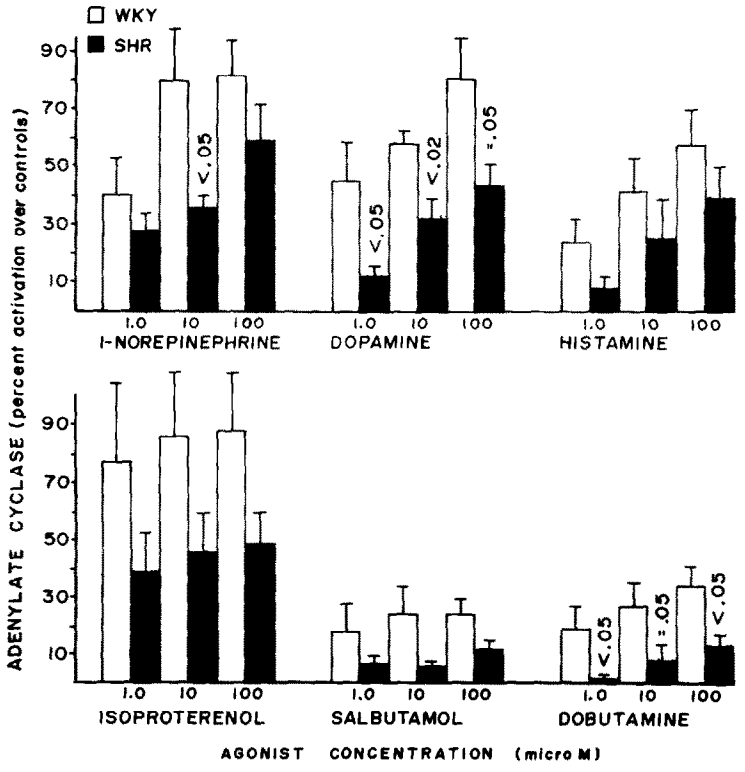

Fig. 1. Actions of neurohumoral agonists on adenylate cyclase in homogenates of frontal cortex from SHR and WKY rats. The values represent the mean ± S.E.M. per cent increases in enzyme activity over respective basal activities. Duplicate assays were run on samples taken from five SHR or five WKY rats. P values (Student's two-tailed *t*-test) are given to denote significantly lowered enzyme responses (per cent activation over basal activity) in SHR when compared to corresponding agonist concentrations in WKY rats. The incubations contained 80 μg of enzyme protein and basal enzyme activities (pmoles cyclic AMP synthesized/min/mg of protein) were: WKY = 87 ± 11, and SHR = 104 ± 10.

as compared to the WKY rats. Basal enzyme activity between the two strains of rats was not appreciably changed (Fig. 2).

The rather generalized reduction in adenylate cyclase responsiveness to the neurohumoral agonists in the frontal cortex and particularly in the cerebral capillaries of the SHR suggests that the receptor defect involved in the hypertensive condition is not specific to any particular subtype. Similar data have been reported for myocardial and vascular tissue in hypertensive rats [14–17]. Moreoever, when SHR were chronically treated for 2 weeks with the centrally acting antihypertensive drug, propranolol, which produces a beta-adrenergic blockade, the adenylate cyclase in the frontal cortex became hypersensitive to stimulation by norepinephrine. During this period, the SHR blood pressure was reduced significantly [19]. Apparently, the chronic adrenergic blockade was responsible for the heightened enzyme sensitivity because Glaubiger and Lefkowitz [21] reported that a similar propranolol treatment yielded an increase in adrenergic receptor density within the rat myocardium. Alternatively, chronic treatment of SHR with the alpha-agonist, clonidine, not only reduced blood pressure again but caused a subsensitivity of cortical adenylate cyclase responses to norepinephrine. Similar investigations using drugs which modify levels of central catecholamines have indicated subtle, as well as prominent, alterations in neurohumoral-induced activation of adenylate cyclase, events coupled to changes in adrenergic receptor density [22]. One possible explanation for the present findings, since the cerebral cortex of SHR is known to contain abnormally high levels of dopamine [23], is that the increased amounts of dopamine released during synaptic activity might induce a compensatory mechanism post-synaptically whereby receptor numbers become subsequently reduced. However, this hypothesis fails to take into account the effects of the beta-adrenergic agonists and histamine in the SHR.

In summary, the present results show a rather non-specific receptor deficit in the neurohumoral activation of adenylate cyclase in both the frontal cortex and cerebral capillaries of the SHR. Before these studies will lead to a better understanding of the pathogenic mechanisms involved in centrally induced hypertension, better animal models will have to be developed. A limited number of investigations have addressed this problem and have used a cross-bred strain (WKY-SHR). However, adenylate cyclase in cardiac tissue of the cross strain was more sensitive to isoproterenol and fluoride than was observed in the individual WKY and SHR [17]. On the other hand, Amer *et al.* [24] reported an attenuated isoproterenol stimulation of adenylate cyclase in both the aortas and hearts of animals made hypertensive with desoxycorticosterone and stress. Thus, development of better animal models and employment of receptor analysis with ligand-binding techniques, coupled to adenylate cyclase activation, will be a major consideration in future investigations concerning central mechanisms of hypertension.

Acknowledgements—This work was supported by grants from the NSF (PCM 77-01995) and the American Heart Association, Alabama Affiliate Inc. Appreciation is expressed to S. Jo Palmer for technical help.

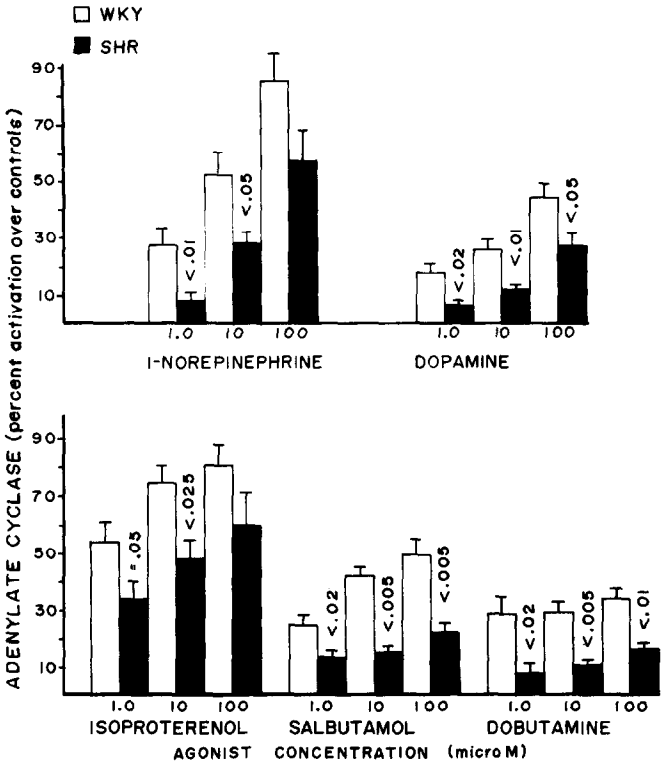

Fig. 2. Actions of neurohumoral agonists on adenylate cyclase in homogenates of isolated cerebral capillaries from SHR and WKY rats. Conditions were the same as described in the legend of Fig. 1 except that six studies from each group were used. Basal enzyme activities were: WKY = 34 ± 3.7, and SHR = 37 ± 5.1; all the samples contained 60 μg of enzyme protein.

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