tically mediated. This suggestion agrees with our finding that reserpine neither reduces phosphodiesterase activity nor increases adenylate cyclase activity when added to adrenal homogenates in concentrations up to $10^{-4}M$ (*15*). Since the splanchnic nerve is cholinergic, the transsynaptic activation of TH implies that acetylcholine may regulate the medullary adenylate cyclase system. Indeed, carbamylcholine, a cholinomimetic drug, increases cyclic AMP concentrations and TH activity in intact and denervated adrenal medulla. The data obtained with aminophylline give further support to the hypothesis that cyclic AMP and TH are both transsynaptically regulated. We tested the inhibition of phosphodiesterase activity by aminophylline in vitro in intact and denervated adrenal medulla and we found that the ID_{50} (dose causing 50 percent inhibition) is equal in both tissues ($10^{-3}M$). Therefore, the accumulation of medullary cyclic AMP elicited by aminophylline injection reflects the in vivo activity of adenylyl cyclase and probably the turnover rate of cyclic AMP in this tissue. Since the rate of medullary cyclic AMP accumulation elicited by aminophylline is greatly reduced by splanchnicotomy, nerve impulses may play a role in the regulation of endogenous cyclic AMP turnover rate in adrenal medulla.

Reserpine (*16*) and carbamylcholine (*5*) release catecholamines from adrenal medulla. It may be inferred that the increase of both cyclic AMP and TH activity elicited by these drugs is in some way related to this release. Several lines of evidence are at variance with the hypothesis that catecholamine release and TH induction are interdependent. Tyramine (130 μmole/kg, intraperitoneally) releases catecholamines from noradrenergic neurons because it reduces by 40 percent the catecholamine concentrations in heart tissue (*17*); although this drug fails to change the steady-state concentrations of adrenal catecholamines it increases their turnover rate and, therefore, it might release catecholamines from adrenal medulla (*17*). However, tyramine increases neither the concentration of cyclic AMP (Fig. 1) nor the TH activity of adrenal glands. Aminophylline changes neither the concentration nor the turnover rate of catecholamines in adrenal medulla (*17*) but it increases cyclic AMP concentration and TH activity of this tissue. Thus, the results reported are consistent with the working hypothesis that an early change in the rate of cyclic AMP accumulation brings about an increase of TH activity. In conclusion, the changing rates of cyclic nucleotide accumulation can be dissociated from a release of adrenal catecholamines but not from the delayed increase of TH activity.

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17. Specific activity of epinephrine, dopamine, and tyrosine was measured (*8*). An injection of [3,5-^{3}H]tyrosine (1 mc/kg, intravenously) was given, and 2 hours later the rats received saline or tyramine (130 μmole/kg, intraperitoneally) or aminophylline (200 μmole/kg, intraperitoneally). They were killed 45 minutes after these injections. Tyramine and aminophylline do not change the steady-state concentration of catecholamines or the specific activity of tyrosine (about 350 dpm/nmole) and dopamine (about 600 dpm/nmole). However, 130 μmole of tyramine per kilogram (intraperitoneally) increases the specific activity of tissue epinephrine (from 53 ± 7 to 77 ± 6 dpm/nmole). At steady state, using the precursor product relationship (*18*), the estimated turnover rate of epinephrine is 0.38 nmole per hour per pair of glands in animals receiving saline and 0.74 nmole per hour per pair of glands in animals receiving tyramine. This dose of tyramine depletes the catecholamine content of heart (from 6.9 ± 0.4 to 4.2 ± 0.6 nmole/g, wet weight, $P < .01$). The turnover rate of adrenal epinephrine of rats receiving aminophylline 45 minutes before is equal to that of controls.
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20 November 1972

Phenylketonuria: Phenylalanine Inhibits Brain Pyruvate Kinase in vivo

Abstract. *The hypothesis that brain damage in phenylketonuria is related to inhibition of pyruvate kinase by phenylalanine was examined in rat brain in vivo. One hour after a single injection of phenylalanine into the rat, the brains were removed and completely frozen in less than a second. The concentration of phenylalanine in the brain was comparable to that found in phenylketonuric patients. Changes in brain glycolytic intermediates were consistent with inhibition of pyruvate kinase in vivo. The inhibition of pyruvate kinase was apparently compensated for by an increase in phosphoenolpyruvate; no decrease in adenosine triphosphate or creatine phosphate was found.*

Phenylketonuria is the most common disorder of amino acid metabolism in man. It is characterized by extraordinarily high levels of phenylalanine and its derivatives in blood and urine (*1*). The basic lesion is the congenital absence of phenylalanine hydroxylase, a hepatic enzyme which converts phenylalanine to tyrosine. Severe mental retardation and motor abnormalities are typical of the untreated disease, but can be prevented by early treatment with a diet low in phenylalanine (*1*). The morphological correlate of the brain damage is a defect in myelination, but the biochemical basis for this abnormality is unclear (*1*).

Weber and co-workers have shown competitive inhibition of purified brain pyruvate kinase (E.C. 2.7.1.40) by phenylalanine (*2*). Phenylalanine also decreased the rate of glycolysis in human and rat brain slices, particularly in those from perinatal rats, in which brain pyruvate kinase activity is relatively low (*2*). These studies have suggested that phenylalanine may have a direct inhibitory effect on energy production in brain, although the relation of the in vitro observations to the mechanism in vivo has not been established (*3*).

We have examined the metabolic effects of hyperphenylalaninemia on

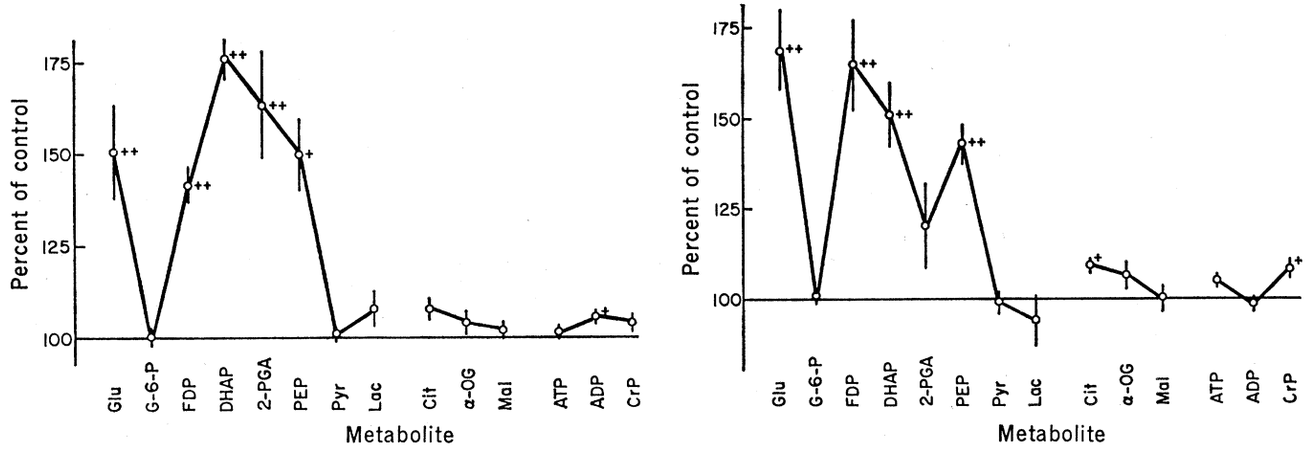

Fig. 1 (left). Metabolite changes in brains of adult rats after phenylalanine. Mean values (± standard error of the mean) of six to ten determinations are plotted as the percentage of the control values [see (*10*)]. The symbols + and ++ indicate statistical significance below the 5 and 1 percent levels, respectively. Abbreviations for metabolites indicate (left to right): glucose, glucose 6-phosphate, fructose 1,6-diphosphate, dihydroxyacetone phosphate, 2-phosphoglyceric acid, phosphoenolpyruvate, pyruvate, lactate, citrate, α-oxoglutarate, malate, adenosine triphosphate, adenosine diphosphate, and creatine phosphate. Fig. 2 (right). Metabolite changes in brains of suckling rats after phenylalanine. Mean values (± standard error of the mean) of ten determinations are plotted as the percentage of the control values [see (*10*)]. Symbols and metabolite abbreviations are the same as in Fig. 1.

brain in vivo. The study was made possible by the development of a new apparatus (*4*) which removes and freezes the brain of the conscious rat within a second, by blowing it out of the cranial vault into a thin layer between two disks previously cooled in liquid nitrogen. The procedure is superior to other methods of freezing the brain or to microwave irradiation because it prevents the postmortem anoxic metabolism that occurs with these methods (*4*). This was of particular importance in these experiments because the concentrations of many glycolytic and Krebs cycle intermediates change rapidly during anoxia [see (*4*, *5*)].

Adult and suckling (20 days postnatal) albino rats were injected with L-phenylalanine (10 mmole/kg, *p*H 9.7, intraperitoneally; Sigma). One hour after injection, the brains were removed and frozen by this new method. Samples were prepared and extracted as described, and metabolites were assayed by standard enzymatic methods (*6*).

At the time the animals were killed, brain phenylalanine in the adult rats was 1.2 μmole/g (*7*), a concentration comparable to the 1 μmole/g found in brains of phenylketonuric patients at autopsy (*8*).

The pattern of brain metabolite changes after the injection of phenylalanine was similar in both age groups (Figs. 1 and 2). The concentrations of glucose and the glycolytic intermediates between glucose 6-phosphate and pyruvate were increased by 40 to 70 percent. Pyruvate, lactate, Krebs cycle intermediates, adenine nucleotides, and creatine phosphate were unaffected by phenylalanine treatment. This pattern is consistent with a partial inhibition of pyruvate kinase, which was overcome by the increase in the concentration of phosphoenolpyruvate. The increase in the other glycolytic intermediates up to fructose 1,6-diphosphate is expected from their linkage to phosphoenolpyruvate through a series of near-equilibrium reactions (*9*). Since the phosphofructokinase step is not near equilibrium, the concentration of glucose 6-phosphate was unaffected by the increased phosphoenolpyruvate.

The increase in brain glucose after phenylalanine treatment could be due to inhibition of brain hexokinase by phenylpyruvate (*3*). Phenylpyruvate concentrations in serum and brain (0.74 m*M* and < 0.05 μmole/g, respectively) were well below those found to cause significant inhibition of brain hexokinase in vitro, however, and increased brain glucose has also been found after treatment with a variety of other agents (*5*, *6*). The mechanism of this effect is therefore uncertain and could be due to altered glucose permeability rather than to a change in hexokinase activity.

The data do not necessarily indicate that a decrease in the rate of glycolysis occurred. In fact the rise in phosphoenolpyruvate would have been sufficient to maintain undiminished flux through the pyruvate kinase reaction, as judged by the degree of inhibition expected from a phenylalanine concentration of 1.2 μmole/g (*3*). Significant impairment of brain glycolysis and energy production might well occur in younger animals continuously exposed to high concentrations of phenylalanine. It is also possible that the increased levels of the glycolytic intermediates preceding pyruvate kinase could produce changes in flux through other pathways and thereby upset the metabolic balance of the brain at crucial times in its development.

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10. Metabolite concentrations (μmole/g) in brains from control groups of six to ten adult rats and ten suckling rats were, respectively: glucose, 1.39 and 1.47; glucose 6-phosphate, 0.146 and 0.141; fructose 1,6-diphosphate, 0.019 and 0.012; dihydroxyacetone phosphate, 0.017 and 0.015; 2-phosphoglyceric acid, 0.0033 and 0.0025; phosphoenolpyruvate, 0.0048 and 0.0035; pyruvate, 0.086 and 0.111; lactate, 1.29 and 1.36; citrate, 0.275 and 0.321; α-oxoglutarate, 0.193 and 0.165; malate, 0.264 and 0.272; adenosine triphosphate, 2.38 and 2.51; adenosine diphosphate, 0.563 and 0.553; creatine phosphate, 3.55 and 3.54.

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Angiotensin II: Important Role in the Maintenance of Arterial Blood Pressure

Abstract. *An angiotensin II antagonist, [1-sarcosine, 8-alanine]-angiotensin II, was given intravenously to anesthetized dogs with thoracic caval constriction and ascites to investigate the role of angiotensin II in the control of arterial pressure. The antagonist produced a striking fall in arterial pressure and in aldosterone secretion and an accompanying increase in plasma renin activity. In a control experiment, normal anesthetized dogs were given the angiotensin analog, but it failed to reduce arterial pressure or to influence plasma renin activity. In conscious dogs with caval constriction, the antagonist produced essentially the same drop in arterial pressure as observed in anesthetized animals. These results suggest an important role for angiotensin II in the maintenance of arterial pressure by its action on specific receptor sites in arteriolar smooth muscle and in the adrenal cortex.*

In 1962, it was discovered that dogs with caval constriction (*1*) or sodium depletion (*1*) and patients with decompensated cirrhosis of the liver (*2*) are less sensitive to synthetic angiotensin II in their pressor response than are normal dogs or normal humans. This phenomenon has never been explained. The experiments reported here were designed to study this problem again and were based on the hypothesis that angiotensin II acts on the peripheral arterioles and plays a role in maintaining blood pressure in these pathophysiological states. It was reasoned that if angiotensin II is displaced from its receptor sites in the smooth muscle of the peripheral arterioles by a competitive antagonist, the arterial pressure will fall. This hypothesis was examined by infusing intravenously an analog of angiotensin II, [1-sarcosine, 8-alanine]-angiotensin II ([Sar^1, Ala^8]-A II), into dogs with thoracic inferior vena cava constriction and ascites. Pals *et al.* (*3*) demonstrated that this compound acts as a competitive antagonist of angiotensin II in the rat.

This angiotensin II antagonist also provides a unique opportunity for evaluating the relative importance of the renin-angiotensin system in the control of aldosterone secretion during caval constriction. Since the renin-angiotensin-aldosterone system is important in the control of sodium excretion and blood volume and thus, indirectly, of blood pressure, it was decided to study the effect of this angiotensin II analog on steroid secretion. A response of a marked decrease in aldosterone secretion would point to an indirect function via blood volume control for angiotensin II in the maintenance of blood pressure.

Under sterile conditions, nine female mongrel hounds were subjected to thoracic inferior vena cava constriction to produce ascites (*4*). Sodium balance studies were conducted while the dogs had a sodium intake of 65 meq/day; every dog showed marked sodium retention and ascites at the time of the experiment. Two days before the study, a catheter was placed in the left adrenolumbar vein (*5*). For the experiment, the dogs were anesthetized with pentobarbital and given 6 mg of dexamethasone (Decadron phosphate, Merck Sharp & Dohme) intramuscularly to depress anterior pituitary function. After control measurements of femoral arterial pressure for 30 minutes and after collection of adrenal venous blood for steroids and external jugular venous blood for plasma renin activity (PRA), [Sar^1, Ala^8]-A II was infused intravenously for 45 minutes at a rate of 6 μg kg^{-1} min^{-1}. Measurements were made at three 15-minute intervals for steroids, and PRA and blood pressure was recorded continuously; two recovery observations were made at 45 and 60

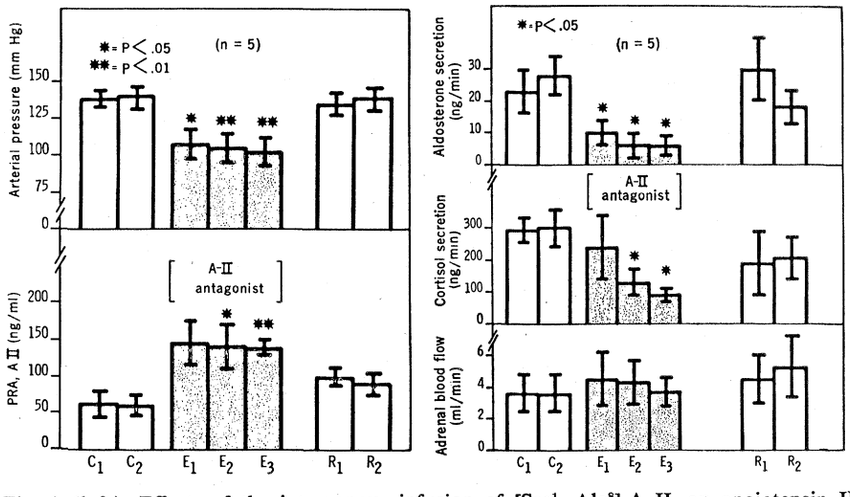

Fig. 1 (left). Effects of the intravenous infusion of [Sar^1, Ala^8]-A II, an angiotensin II (*A-II*) antagonist, on femoral arterial pressure and plasma renin activity (*PRA*) in five dogs with thoracic caval constriction. The abbreviations C_1, C_2, E_1, E_2, E_3, R_1, and R_2 represent control, experimental, and recovery periods. Fig. 2 (right). Effects of the A-II antagonist on aldosterone and corticol secretion and adrenal blood flow in the same dogs with thoracic caval constriction.

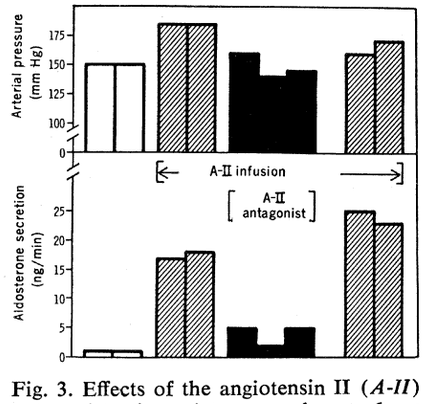

Fig. 3. Effects of the angiotensin II (*A-II*) antagonist given intravenously at 6 μg kg^{-1} min^{-1} on arterial pressure and aldosterone secretion during the simultaneous infusion of A-II at 1.5 μg min^{-1} in a normal dog.