Experiments showed that, when high amplitude impulsation had been abolished by hexonium, the carotid clamping was not followed by the constriction of spleen resistance vessels, while a capacitance vessel response (constriction or dilatation) was persistent in most cases. Venous outflow increased by 4.2 ± 0.8% above control in 50% of experiments, decreased by 6.8 ± 2.9% in 33%, and was unchanged in 17% of experiments.

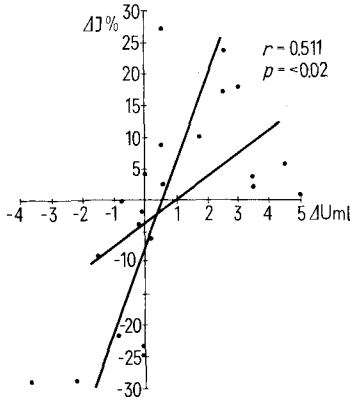

Fig. 2. Direct correlation between changes of venous outflow from spleen vessels and the rate of low amplitude impulses in splenic nerve under pressor carotid sinus reflex after hexonium (2 mg/kg). Abscissa, magnitude of outflow changes in ml (ΔU, ml); ordinate, impulse rate in % to control (ΔI, %).

Changes of the low amplitude impulse rate in the splenic nerve were of the same direction as those of the venous outflow in 79%. The increase of impulse rate (by 8.5 ± 2.4% on the average) in 15% of cases was accompanied by a rise of venous outflow, and in 33.3% of cases decrease of the impulse rate (by 14.3 ± 4.7%) was accompanied by an outflow decrease. These data are summarized in Figure 1.

Correlation and regression analysis showed that a direct correlation existed between the changes of low amplitude impulse rate and the changes of venous outflow ($r = 0.511$, $p < 0.02$): the more the increase of low amplitude impulse rate, the more the magnitude of venous outflow increase; and the more the decrease of the impulse rate, the lesser was the outflow (Figure 2).

Thus, the study of the vasomotor reactions of the spleen with simultaneous recording of the efferent sympathetic activity permits us to suggest that high amplitude impulsation is related to the responses of resistance vessels, and low amplitude impulses, to the responses of capacitance vessels.

'Binding' of Glutamate and Aspartate to Synaptosomal Fractions of Six Regions of the Feline Brain[1]

F. V. DE FEUDIS, P. MADTES, A. OJEDA and M. GADEA-CIRIA

Departamento de Investigación, Centro Nacional 'Ramon y Cajal' (de la Seguridad Social) y Facultad de Medicina Autónoma, Arzobispo Morcillo 1, Madrid 34 (Spain), 6 February 1976.

Summary. The order of potency of 'binding' of both glutamate and aspartate to synaptosomal fractions of brain regions was: cerebellar cortex ⩾ caudate nucleus ≧ cerebral cortex > medulla ≅ pons > corona radiata. Glutamate was bound to a greater extent than aspartate to particles of all regions studied, except for cerebral cortex.

L-Glutamate and L-asparate, leading candidates for roles as excitatory neurotransmitters, are the most potent and quickest-acting excitatory agents present in the mammalian CNS[2-4]. Since 'high'- and 'low-affinity' uptake processes for these amino acids in various CNS regions[5-7] and their subcellular distributions[8] are very similar, we have devised a simple method by which regional differences in their 'binding' can be demonstrated, as has been shown for GABA, glycine and taurine[9-13].

Materials and methods. Adult male cats (2.8–3.3 kg) were anesthetized with pentobarbitone-Na+ (30 mg/kg) and then killed by air embolism. Regions of the brain were dissected out on a chilled, moist surface, weighed, and homogenized in 10 volumes of ice-cold isosmotic (0.32 *M*) sucrose solution. All further operations were conducted at 0 °C. The subcellular fractionation procedure employed was essentially that of GRAY and WHITTAKER[14], as modified[10], except that the 'binding' of L-glutamate and L-aspartate to synaptosome-enriched fractions was examined in a balanced, bicarbonate-buffered medium, rather than in sucrose solutions. The osmolarities of the ions present in this glucose free medium were; Na^+, 147.3; K^+, 3.5; Ca^{++}, 1.3; Mg^{++}, 1.2; Cl^-, 128.5; HCO_3^-, 24.55; PO_4^{---}, 0.45 and SO_4^{--}, 1.2 mOsmoles/liter; total osmolarity ≅ 308 mOs*M*; pH was 7.4 after equilibration with 95% O_2/5% CO_2.

Portions (4.0 ml) of homogenates representing 0.364 g tissue, were centrifuged at 1000 × *g*, 10 min to prepare first supernatant (S_1) fractions. Centrifugation of 3.0 ml portions of S_1 fractions at 17,000 × *g*, 30 min provided synaptosomal mitochondrial (P_2) fractions, which were re-suspended in 3.0 ml of 0.32 *M* sucrose solution and re-centrifuged at 17,000 × *g*, 30 min. Washed P_2 fractions were re-suspended in 3.0 ml of physiological medium,

[1] Acknowledgments. This study was supported by Centro Nacional 'Ramon y Cajal' and Fundación Juan March. Thanks are due to Professor J. M. R. DELGADO for organizing research facilities and for reading this manuscript, and to Srta. FRANCISCA MOYA and Srta. AMPARO LATORRE for technical assistance.

[2] F. V. DEFEUDIS, A. Rev. Pharmac. *15*, 105 (1975).
[3] K. KRNJEVIĆ, in *Excitatory Synaptic Mechanisms* (Universitetsforlaget, Oslo 1970), p. 95.
[4] K. KRNJEVIĆ, Physiol. Rev. *54*, 418 (1974).
[5] V. J. BALCAR and G. A. R. JOHNSTON, J. Neurochem. *20*, 529 (1973).
[6] W. J. LOGAN and S. H. SNYDER, Brain Res. *42*, 413 (1972).
[7] M. J. NEAL and R. D. WHITE, Br. J. Pharmac. *49*, 172P (1973).
[8] A. R. WOFESY, M. J. KUHAR and S. H. SNYDER, Proc. natn. Acad. Sci., USA *68*, 1102 (1971).
[9] F. V. DEFEUDIS, Can. J. Physiol. Pharmac. *51*, 873 (1973).
[10] F. V. DEFEUDIS, Can. J. Physiol. Pharmac. *52*, 138 (1974).
[11] F. V. DEFEUDIS and W. C. BLACK, Brain Res. *49*, 218 (1973)
[12] F. V. DEFEUDIS and N. SCHIFF, Expl Neurol. *48*, 325 (1975).
[13] M. GADEA-CIRIA, M. GARCÍA-GRACÍA, J. GERVAS-CAMACHO, E. SOMOZA, G. BALFAGÓN and F. V. DEFEUDIS, J. Neurosci. Res., *1*, 393 (1975).
[14] E. G. GRAY and V. P. WHITTAKER, J. Anat. *96*, 79 (1962).

and 250 µl aliquots of these suspensions were pipetted into centrifuge tubes. Then, 250 µl of physiological medium were added and the samples were mixed and allowed to stand for 10 min at 0 °C. Lastly, 0.5 ml of a radioactive solution which provided final concentrations of 2.1×10^{-7} *M* of U-^{14}C-L-glutamate (New England Nuclear Corp.; 234 mCi/mmole) and 2,3-^{3}H-L-aspartate (New England Nuclear Corp.; 26 Ci/mmole) were added, and the samples were mixed and allowed to stand at 0 °C for 15 min before final centrifugation at $17{,}000 \times g$, 30 min. Pellet and supernatant fractions were prepared for determination of radioactivity[10,15]. The use of 0.5×10^{-6} *M* ^{14}C-sucrose (New England Nuclear Corp.; 505 mCi/mmole) in the media in identical experiments permitted correction for the amounts of ^{3}H and ^{14}C entrapped in supernatant fluid of the pellets. All data were corrected for the amounts of amino acids present as occluded supernatant fluid of the pellets[10,16].

'Binding' of glutamate and aspartate to synaptosomal fractions of 6 regions of the feline brain

Brain region	nmole/g P_2 in non-sucrose space	
	^{14}C-glutamate	^{3}H-aspartate
Cerebellar cortex	10.7 ± 0.5 [b]	7.1 ± 0.4
Cerebral cortex	3.2 ± 0.1	3.2 ± 0.2
Pons	2.7 ± 0.05 [b]	1.9 ± 0.04
Medulla oblongata	2.9 ± 0.06 [b]	2.0 ± 0.05
Caudate nucleus	3.9 ± 0.1 [a]	3.3 ± 0.15
Corona radiata	1.6 ± 0.1 [a]	1.2 ± 0.1

Final concentrations of ^{14}C-glutamate and ^{3}H-asparate in the medium were 2.1×10^{-7} *M* (see Methods); radioactivity was determined by a double-isotope method[10]. Means ± SEM; 17 or 18 tissue samples in all cases; [a] and [b] indicate, respectively, $p < 0.01$ and $p < 0.001$, when these values are compared with corresponding values for ^{3}H-aspartate; all values for the 'binding' of ^{14}C-glutamate and ^{3}H-aspartate to particles of cerebellar cortex were significantly greater ($p < 0.001$) than those for all other regions (Student's *t*-test; two tailed).

Results and discussion. Results shown in the Table indicated that both amino acids were bound to a considerable extent to particles prepared from all regions of the brain that were studied, and that pronounced regional differences existed in this 'binding'. The order of potency of 'binding' of glutamate and aspartate to the particles was; cerebellar cortex ⩾ caudate nucleus ≧ cerebral cortex > medulla oblongata ≅ pons > corona radiata. Glutamate was bound to a greater extent than aspartate to particles of all regions studied, except for cerebral cortex. This study has revealed that significant differences exist in the 'binding' of glutamate and aspartate to synaptosome-enriched fractions of some cerebral structures. These differences, like those observed in studies of the 'binding' of GABA and glycine to synaptosomal fractions of cerebral cortex and spinal cord[9,10], cannot be explained simply by differences which exist in the endogenous tissue contents of these amino acids, and consequently, they could be related to the physiological actions of these amino acids, e.g., to mechanisms of transmitter-inactivation ('re-uptake') and/or receptor-interaction. It is noteworthy that the results presented herein revealed regional differences that could not be shown clearly by kinetic studies of the uptake processes for these amino acids, e.g.,[5,6]. Such differences might even become more pronounced if binding studies are carried out in the absence of Na^{+} (see ref.[17]).

[15] F. V. DeFeudis, Res. Commun. chem. Path. Pharmac. *2*, 189 (1971).

[16] F. V. DeFeudis, G. Balfagón, M. R. de Sagarra, P. Madtes, E. Somoza and J. Gervas-Camacho, Expl Neurol. *49*, 497 (1975).

[17] N. A. Peterson and E. Raghupathy, Biochem. Pharmacol. *23*, 2491 (1974).

Deficiency in Renomedullary Prostaglandin Synthesis Related to the Evolution of Essential Hypertension

N. Papanicolaou[1], Th. Mountokalakis, M. Safar, J. Bariety and P. Milliez

Centre de Recherche sur l'Hypertension Artérielle, Institut National de la Santé et de la Recherche Médicale (U-28), Hôpital Broussais, 96, rue Didot, F-75014 Paris (France), 21 January 1976.

Summary. Continued PG synthesis in the early stages of essential hypertension might reflect an activation of the renal antihypertensive function in respect to neurogenic and/or hormonal pression stimuli, subsequently a deficiency of renal PG synthesis related to irreversible changes with the kidney would lead to the prepoderance of a pressure mechanism, resulting to a further increase of blood pressure.

The identification of the potent antihypertensive prostaglandins (PGs) in the renal medulla[2,3] has provided further support for the concept that states of animal and human hypertension may result from a deficiency of renal depressor systems[4]. Since natural PGs occurring in the urine have been thought to reflect renal PGs synthesis[5], the detection of urinary PGs in patients with arterial hypertension could serve as a useful tool for testing this hypothesis. We therefore decided to invesigate the presence of substances with chromatographic behaviour and the bioassay properties of PGs in the urine of a group of patients with essential hypertension. The results are suggestive of a deficiency in renomedullary PG synthesis related to the evolution of the hypertensive disease.

Patients and methods. 21 patients with essential hypertension, 15 males and 6 females, aged 19 to 53

[1] We thank Dr. J. R. Pike of the Upjohn Company, Kalamazoo Michigan, who kindly provided PGs. This work was supported by a grant from INSERM (ATP 17 No. of contract *735479 17*) to Dr. Papanicolaou.

[2] K. Crowshaw, J. McGiff, J. Strand, A. Lonigro and N. Terragno, J. Pharm. Pharmac. *22*, 302 (1970).

[3] N. Papanicolaou, S. Makrakis, J. Bariety and P. Milliez, J. Pharm. Pharmac. *26*, 270 (1974).

[4] J. Lee, Arch. intern. Med. *133*, 56 (1974).

[5] J. Frölich, B. Sweetman, K. Carr, J. Splawinski, J. Watson, E. Anggard and J. Oates, *Advances in Biosciences* (Ed. S. Bergström; Pergamon Press, New York 1973), vol. 9, p. 321.