REFERENCES

BLONDAUX, C., JUGE, A., SORDET, F., CHOUVET, G., JOUVET, M. & PUJOL, J. F. (1973). *Brain Res.*, **50**, 101–114.
CURZON, G. & GREEN, A. R. (1970). *Br. J. Pharmac.*, **39**, 653–655.
D'AMICO, D. J., PATEL, B. C. & KLAWANS, H. L. (1976). *J. Pharm. Pharmac.*, **28**, 454–456.
FREEDMAN, D. X. & BOGGAN, W. O. (1974). *Adv. Biochem. Psychopharmac.*, **10**, 151–157.
FULLER, R. W. & PERRY, K. W. (1974). *Brain Res.*, **70**, 369–371.
FULLER, R. W., SNODDY, H. D., PERRY, K. W., ROUSH, B. W., MOLLOY, B. B., BYMASTER, F. R. & WONG, D. T. (1976). *Life Sci.*, **18**, 925–934.
GRABOWSKA, M. (1974). *Pol. J. Pharmac. Pharm.*, **26**, 499–503.
GRABOWSKA, M. (1975). *Pharmac. Biochem. Behav.*, **3**, 589–591.
GRABOWSKA, M., ANTKIEWICZ, L. & MICHALUK, J. (1974a). *Biochem. Pharmac.*, **23**, 3211–3213.
GRABOWSKA, M., ANTKIEWICZ, L. & MICHALUK, J. (1974b). *J. Pharm. Pharmac.*, **26**, 74–76.
GREEN, A. R. & GRAHAME-SMITH, D. G. (1976). *Nature*, **262**, 594–596.
GREEN, A. R., YOUDIM, M. B. H. & GRAHAME-SMITH, D. G. (1976). *Neuropharmacology*, **15**, 173–179, 1976.
HAIGLER, H. J. & AGHAJANIAN, G. K. (1974). *J. Neural. Trans.*, **35**, 257–273, 1974.
HAMON, M., BOURGOIN, S., ENJALBERT, A., BOCKAERT, J., HERY, F., TERNAUX, J. P. & GLOWINSKI, J. (1976). *Naunyn-Schmiedebergs Arch. Pharmac.*, **294**, 99–108.
JACOBY, J. H. & BRYCE, G. F. (1976). *Society for Neurosciences* (6th Annual Meeting), Abstract 700.
JACOBY, J. H., HOWD, R. A., LEVIN, M. S. & WURTMAN, R. J. (1976). *Neuropharmacology*, **15**, 529–534.
JACOBY, J. H. POULAKOS, J. J. & BRYCE, G. F. (1977). *Neuropharmacology*, in the press.
JACOBY, H. J., SHABSHELOWITZ, H., FERNSTROM, J. D. & WURTMAN, R. J. (1975). *J. Pharmac. exp. Ther.*, **195**, 257–264.
JACOBY, J. H., POULAKOS, J. J. & BRYCE, G. F. (1977). *Neuropharmacology*, in the press.
JOHNSON, G., KIM, E. & BOUKMA, J. J. (1972). *Ibid.*, **180**, 539–546.
KELLY, P. H. & IVERSEN, L. L. (1975). *Psychopharm.*, **45**, 221–224.
KOSTOWSKI, W., SAMANIN, R., BAREGGI, S., MARC, V., GARATTINI, S. & VALZELLI, L. (1974). *Brain Res.*, **82**, 178–182.
LLOYD, K. G. & BARTHOLINI, G. (1974). *Adv. Biochem. Psychopharm.*, **10**, 305–309.
MEDON, P. J., LEELING, J. L. & PHILLIPS, B. M. (1973). *Life Sci.*, **13**, 685–691.
MIDDLEMISS, D. N., BLAKEBOROUGH, L. & LEATHER, S. R. (1977). *Nature*, **267**, 289–290, 1977.
MONACHON, M. A., BURKARD, W. P., JALFRE, J. & HAEFELY, W. (1972). *Naunyn-Schmiedebergs Arch. Pharmac.*, **274**, 192–197.
MOSKO, S. S. & JACOBS, B. L. (1977). *Brain Res.*, **119**, 291–303.
PIERI, L., PIERI, M. & HAEFELY, W. (1974). *Nature*, **252**, 586–588.
ROSECRANS, J. A., LOVELL, R. A. & FREEDMAN, D. X. (1967). *Biochem. Pharmac.*, **16**, 2011–2021.
SOFIA, R. D. & VASSAR, H. B. (1975). *Archs int. Pharmacodyn. Thér.*, **216**, 40–50.
STEIN, D., JOUVET, M. & PUJOL, J. F. (1974). *Brain Res.*, **72**, 360–365.
VON HUNGEN, K., ROBERTS, S. & HILL, D. F. (1974). *Nature*, **252**, 588–589.

2, 4-Dinitrophenol inhibition of transport of 5-hydroxyindoleacetic acid from the cerebrospinal fluid and spinal cord

BRANIMIR ŽIVKOVIĆ, MARIN BULAT*, *Institute 'Rudjer Boškovic', Bijenička c. 54, 41001 Zagreb, Yugoslavia*

5-Hydroxyindoleacetic acid (5-HIAA) in the lumbar cerebrospinal fluid (csf) of patients is often analysed to obtain insight into metabolism of 5-hydroxytryptamine in the central nervous system. Recent experiments indicate that this acid in the lumbar csf is derived from the adjacent spinal cord (Bulat, Lacković & others, 1974; Jakupčević, Lacković & others, 1977) rather than from the brain (Bulat, 1977) or blood (Bulat & Živković, 1973). Probenecid is a competitive inhibitor of 5-HIAA transport from the spinal cord (Bulat, 1974), lumbar csf and cisternal csf (Živković & Bulat, 1971; Wolfson, Katzman & Escriva, 1974). To find if this transport of 5-HIAA requires metabolic energy derived from ATP we treated cats with 2,4-dinitrophenol which inhibits the formation of ATP by uncoupling oxidative phosphorylation (Davson, 1967).

Adult cats (2·5–3·5 kg) of either sex were lightly anaethetised with thiopentone sodium anaesthesia (50 mg kg^{-1}, i.p.). Laminectomy was performed at the lumbar (L5-L7 vertebrae) and thoracic (T11 vertebra)

* Correspondence.

regions. An extradural ligature at T11 region, pressing gently on the spinal cord, was positioned to prevent any potential mixing of the csf below and above the ligature (Živković & Bulat, 1971). One group of animals was treated with dinitrophenol (30 mg kg^{-1}, i.p.) and another group (control) with saline. Seventy five min after the treatment, samples of csf were obtained by puncture of the cisterna magna (cisternal csf; 0·80 ml) and the exposed lumbar subarachnoid space (lumbar csf; 0·50 ml). The spinal cord was transected at T11 region and its caudal segment taken for analysis. 5-HIAA in samples of csf and spinal cord was determined according to Ashcroft & Sharman (1962).

After administration of dinitrophenol a significant increase of 5-HIAA in the spinal cord, lumbar and cisternal csf is observed (Table 1), the increase in lumbar csf being about twice that in the spinal cord (Table 1). A similar phenomenon was found after application of probenecid (our unpublished results). In animals treated with dinitrophenol (30 mg kg^{-1}, i.p.; Table 1) an increase or rectal temperature (Reid, Volicer & Brodie, 1968) was also observed (3·37 ± 0·55°). In preliminary experiments the lower doses of dinitrophenol (20 or 25 mg kg^{-1}, i.p.) failed to increase both the concentration of 5-HIAA in the csf and rectal temperature.

Since after dinitrophenol application there is an increase in 5-HIAA in the csf and spinal cord, this indicates that ATP is required for the operation of the carrier system which transports 5-HIAA from these compartments. It was shown previously (Reid, Volicer & Brodie, 1968) that dinitrophenol inhibits the transport of 5-HIAA from rat brain. Thus, it appears that its transport from the csf and the central nervous system is an active ATP-dependent process.

Table 1. *Concentration of 5-HIAA in the spinal cord, lumbar csf and cisternal csf 75 min after application of dinitrophenol (30 mg kg^{-1}, i.p.) or saline (control).*

	5-HIAA (ng g^{-1} or ng ml^{-1}) Saline	DNP	Increase %	*t*-test
Spinal cord	136 ± 13(6)*	183 ± 8(5)	135	$P < 0.05$
Lumbar csf	104 ± 5(4)	185 ± 24(4)	178	$P < 0.05$
Cisternal csf	120 ± 10(4)	188 ± 11(4)	157	$P < 0.01$

* Mean ± standard error of the mean,
Figures in parentheses represent number of cats,

To explain the increase of 5-HIAA in the lumbar csf above that in the spinal cord after dinitrophenol treatment (Table 1), an unequal distribution of 5-HIAA in the various compartments of the spinal cord has to be postulated. Since it is ionized at physiological pH (Neff, Tozer & Brodie, 1967), its penetration from the spinal extracellular space into a large cellular compartment (neurons, glia) should be restricted. On the contrary, its diffusion from the extracellular space into lumbar csf seems to be relatively free (Bulat, 1974). Thus, after inhibition by dinitrophenol of the active transport of 5-HIAA from the extracellular space and lumbar csf to the bloodstream, a high accumulation of 5-HIAA in the extracellular space and consequently in the lumbar csf should be expected. However, since we cannot determine its concentration in the small extracellular space separately but only together with the large cellular compartment which contains low concentration of 5-HIAA, the increase of 5-HIAA in the total spinal tissue should be smaller than that in the spinal extracellular space and lumbar csf.

Supported by Fund for Scientific Activities of SR Croatia and PL 480 Research Agreement No. 01-015-1.

July 20, 1977

REFERENCES

ASHCROFT, G. W. & SHARMAN, D. F. (1962). *Br. J. Pharmac.*, **19**, 153–160.

BULAT, M. (1974). *Aromatic amino acids in the brain*, Ciba Foundation Symposium 22, pp. 243–264, Amsterdam: Elsevier.

BULAT, M. (1977). *Brain Res.*, **122**, 388–391.

BULAT, M., LACKOVIĆ, Z., JAKUPČEVIĆ, M. & DAMJANOV, I. (1974). *Science*, **1-5**, 526–528.

BULAT, M. & ŽIVKOVIĆ, B. (1973). *J. Pharm. Pharmac.*, **25**, 178–179.

DAVSON, H. (1967). *Physiology of the cerebrospinal fluid*, p. 137, London: Churchill Ltd.

JAKUPČEVIĆ, M., LACKOVIĆ, Z., STEFOSKI, D. & BULAT, M. (1977). *J. neur. Sci.*, **31**, 165–171.

NEFF, N. H. TOZER, T. N. & BRODIE, B. B. (1967). *J. Pharmac. exp. Ther.*, **158**, 214–218.

REID, W. D., VOLICER, L. & BRODIE, B. B. (1968). *Life Sci.*, **7**, 577–581.

WOLFSON, L. I., KATZMAN, R. & ESCRIVA, A. (1974). *Neurology*, **24**, 772–779.

ŽIVKOVIĆ, B. & BULAT, M. (1971). *J. Pharm. Pharmac.*, **23**, 539–540.