On the Effect of Amantadine on ATP Content and ATPase Activity in Brain and Blood of Rats

A considerable experimental and clinical material has been collected on the action of amantadine as an antiparkinsonian agent[1–4], but little is known about the exact mechanism of the drug in parkinsonian patients[5–11]. The clinical observations have revealed that amantadine, when administered prior to or simultaneously with L-DOPA, increases the effect and utilization of L-DOPA[12–14]. We suggested that this property of amantadine might be the result of an improved cell membrane permeability, which depends on K-Na-ATPase, the latter hydrolyzing ATP (for the supply of energy). Moreover, ATP together with a protein participates in the storage of catecholamines (CA) in granules[15–17], while ATPase plays an important role in the spontaneous liberation of the amines from the vesicles during excitement[15]. Investigations on the adrenergic nerve endings, carried out in vitro, have shown that the storage of CA depended exclusively on ATP and ATPase[18]. With the purpose of elucidating the mechanism of action of amantadine as an antiparkinsonian agent, we studied the effect of amantadine-HCl on ATP content and ATPase activity. In addition, investigation and comparison were made of the interaction of amantadine with reserpine, which induces a depletion of monoamines from the amine granules[19–23].

Material and methods. Adult rats of 200–220 g body wt. were twice injected i.p. with 50 mg/kg, and others with 100 mg/kg amantadine. Other groups of animals were treated chronically s.c. for 10–15 days with 1–2 mg/kg of reserpine. After the development of parkinsonian symptomatology, the latter group was divided into 2 subgroups. The rats from the 1st subgroup were killed and examined, while those of the 2nd subgroup were treated with amantadine, 50 or 100 mg/kg for 10–12 days. A 3rd group of rats were simultaneously treated with reserpine and amantadine in the same doses for 10 days. Control rats were injected with the same doses of saline daily. The rats were killed 60–80 min after the last injection. The head of the killed animal was dropped into liquid nitrogen, after which the brain was taked out. Biochemical analyses of ATP were carried out with biochemical test combination Boehringer by the method of ADAM[24]. K-Na-ATPase activity was measured in an incubation medium of 30 m*M* *tris*-HCl with pH = 7.4, 10 m*M* NaCl, 30 m*M* KCl, 6 m*M* $MgCl_2$ 3 m*M* ATP and 0.1 ml of 10% homogenate with buffer *tris*-HCl. ATPase activity was estimated from the excretory amounts of inorganic phosphate by an incubation period of 10 min, and photometrically analyzed in a spectrophotometer Opton at 720 nm. The protein content was estimated by the biuret method.

[1] R. S. SCHWAB, A. C. ENGLAND, D. C. POSKANZER and R. R. YOUNG, J. Am. med. Ass. *208*, 1168 (1969).
[2] J. D. PARKES, M. D. KALER, K. J. ZILKHA and R. P. KNILL-JONES, Lancet *7*, 259 (1970).
[3] J. D. PARKES, PH. MARSDEN, K. J. ZILKHA, R. P. KNILL and R. C. H. BAXTER, Lancet *7*, 30 (1970).
[4] G. W. VÖLLER, Klin. Wschr. *17*, 934 (1970).
[5] B. SCATTON, A. CHERAMY, M. J. BESSON and J. GLOWINSKI, Eur. J. Pharmac. *13*, 131 (1970).
[6] U. STRÖMBERG and T. H. SVENSSON, Acta pharmac. Copenh. *30*, 161 (1971).
[7] R. P. GRELAK, R. CLARK, J. M. STUMP, V. G. VERMER, Science *169*, 203 (1970).
[8] P. F. VON VOITLÄNDER and K. E. MOORE, Science *174*, 408 (1971).
[9] U. K. RINNE, V. SONNINEN and M. HYYPPÄ, Experientia Basel *28*, 57 (1972).
[10] GY. MOLNAR and A. FODOR, Semmelweis med. Univ., Budapest *2214*, 1–14 (1971).
[11] I. J. BAK, R. HASSLER, J. S. KIM and K. KATAOKA, J. Neural Transmiss. *33*, 45 (1972).
[11] I. J. BAK, R. HASSLER, J. S. KIM and K. KATAOKA, J. Neural Transmiss. *33*, 45 (1972).
[12] D. C. MANN, L. A. PLARCE, L. D. WATERBURY, Neurology *21*, 958 (1971).
[13] R. B. GODWIN-AUSTEN, C. C. FREARS, S. BERGMAN and R. P. KNILL-JONES, Lancet *2*, 383 (1970).
[14] W. H. TIMBERLAKE, Neurology *20*, 31 (1970).
[15] N. A. HILLARP and B. WALDECK, Acta physiol. scand. *59*, Suppl., 215 (1963).
[16] A. PLETSCHER, M. DA PRADA, K. H. BERNEIS and J. P. TRANZER, Abstracts 70, 7th Meet. FEBS, Varna (1971).
[17] A. CARLSSON, Pharmac. Rev. *18*, 541 (1966).
[18] M. GAJÓ, G. KÁLMÁN, K. GARAY und B. CSILLIK, J. neural-Visc. Relations *32*, 11 (1970).
[19] A. CARLSSON, E. ROSENGREN, Å. BERTLER and J. NILLSSON Psychotropic Drugs (Elsevier Publisher Comp., Amsterdam 1957), p. 363.
[20] A. DAHLSTRÖM, K. FUXE and N. Å. HILLARP, Acta pharmac. Copenh. *22*, 277 (1965).
[21] A. DAHLSTRÖM and K. FUXE, Acta physiol. scand. *62*, Suppl. 232 (1964).
[22] W. BIRKMAYER and O. HORNYKIEWICZ, Wien. klin. Wschr. *73*, 787 (1961).
[23] H. EHRINGER and O. HORNYKIEWICZ, Klin. Wschr. *38*, 1236 (1960).
[24] H. ADAM, in *Methods in Enzymatic Analysis*, 2nd edn. (Ed. H. U. Bergmeyer; Verlag Chemie, Weinheim 1965), p. 539.

ATP content (m*M*/g body wt.) and K^+-Na^+-ATPase activity (µg/P/mg protein/h) in brain and blood of rats, treated with amantadine. HCl and Reserpine (1–2 mg/kg)

Rats	No. experiments	mg ATP/ 100 ml blood	ATP content		K-Na-ATPase activity	
			Brain stem	Hemispheres	Brain stem	Hemispheres
Control	10	19.23	1.620 ± 0.128	1.430 ± 0.118	81.44 ± 0.24	57.32 ± 0.92
Amantadine (50 mg/kg)	10	32.46[a]	1.602 ± 0.112	1.413 ± 0.101	99.41 ± 0.68[a]	56.08 ± 0.98
Amantadine (100 mg/kg)	10	34.22[a]	1.438 ± 0.082	1.328 ± 0.117	102.48 ± 0.84[a]	58.02 ± 1.08
Reserpine	8	10.82[a]	1.038 ± 0.098[a]	1.402 ± 0.192	77.15 ± 0.32	56.98 ± 1.15
Reserpine + Amantadine (100 mg/kg)	7	18.28	0.892 ± 0.093[a]	1.128 ± 0.089	122.14 ± 0.92[a]	70.20 ± 1.16
Amantadine (50 mg/kg) by chr. reserp.	9	22.41[c]	1.070 ± 0.205[a]	—	80.42 ± 0.46	—
Amantadine (100 mg/kg) by chr. reserp.	8	23.61[b]	1.098 ± 0.099[a]	—	83.49 ± 0.73	—

The differences between control and experimental groups were assessed by *t*-test. [a] p 0.001. [b] p 0.01. [c] p 0.05.

Results. The administration of amantadine alone in a dose of 100 mg/kg induced consequently parkinsonian symptoms, such as tremor and humpback followed by an increased motor activity. When injected simultaneously with reserpine, amantadine aggravated all manifestations of the model of reserpine parkinsonism in rats. When administered with a therapeutic purpose on the background of parkinsonian symptomatology previously induced by chronic reserpinization, amantadine delayed the persistence of the parkinsonian symptoms in the animals.

The Table shows that amantadine in doses of 50 and 100 mg/kg increased K-Na-ATPase activity in the stem. The ATP content and ATPase activity in the stem of chronically reserpinized animals were significantly decreased as compared with the controls. The animals treated simultaneously with reserpine and amantadine exhibited a significantly decreased ATP content and an increased ATPase activity. As can be seen from the Table, the amantadine therapy of chronically reserpinized animals led to normalization of ATPase activity and to a more difficult restoration of ATP content in the brain stem. The amantadine increases, while reserpine decreases the blood ATP level.

Discussion. The results of our investigations on rats treated with amantadine point to an increased K-Na-ATPase activity. A still higher activity is found in the brain stem of animals given simultaneous chronic treatment with reserpine and amantadine. This gives us reason to consider that the mechanism of amantadine consists in an action on the transfer mechanisms in which the ouabain sensitive K-Na-ATPase takes an active part. We found a decreased ATPase activity and ATP content in the brain stem of chronically reserpinized rats. The storage of CA in the amine granules is shown by means of a mechanism, which is dependent on ATP and ATP-ase[18–21]. The decreased ATP content and increased ATP-ase activity in the brain stem of the animals treated with reserpine and amantadine, explain also to a great extent the pharmacological potentiation of the reserpine effect by amantadine. The reduced content of the binding agent – ATP, and the increased membrane permeability (raised K-Na-ATPase), lead to an enhanced membrane transfer and intense release of CA[25].

Amantadine increases the total blood ATP level in these animals. This might also be evidence in support of our view for the mechanism of action of amantadine upon cell permeability and the storage of CA in the blood cells.

It seems likely that the good therapeutic effect of amantadine alone in parkinsonian patients is due to its property to exert an influence on the transfer mechanisms, which are disturbed in parkinsonism[25]. The results of our investigations make us believe that the amantadine effect consists in an increase of cell membrane penetration.

Zusammenfassung. Nachweis, dass Amantadin-HCl die Aktivität der K-Na-ATPase im Hirnstamm von Ratten erhöht. Für diese Wirkung bei extrapyramidalen Syndromen wird die Veränderung der Membranpermeabilität verantwortlich gemacht.

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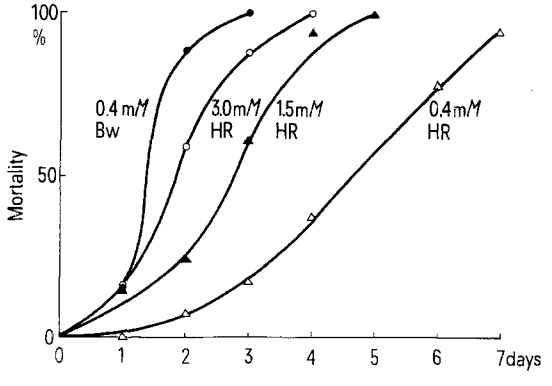
[25] V. VELKOV, Acta morph., Brno *21*, 345 (1973).

Mutagenic Activity of the Insecticide Oxydemetonmethyl in a Resistant Strain of *Drosophila melanogaster*

Oxydemetonmethyl (0,0-dimethyl-S-2-(ethylsulfinyl)-ethyl phosphorothioate) is widely used as a systemic and contact insecticide for the control of insects and mites It has a range of action similar to that of demetonmethyl of which it is a metabolic product[1]. It has been suggested that this insecticide, which has been proved to be a monofunctional alkylating agent, causes forward mutations in *Escherichia coli*[2] and produces trp-conversions in *Saccharomyces cerevisiae*[3]. Therefore, we were interested in whether this compound might also be mutagenic in a higher organism like *Drosophila*.

The strains used were Berlin wild and Hikone R (HR); HR shows resistance to a series of insecticides such as DDT, chlordane, parathion, and dinitrocresol (DNOC)[4,5]. It is, however, highly susceptible to phenylthiourea[6]. Both mortality and the induction of X-linked recessive lethals as a function of dose were compared by treating 1-2-day-old adult males of both strains using the adult feeding technique described in detail elsewhere[7].

Oxydemetonmethyl was dissolved in 5% sucrose solution and fed to the adult males for either 17 h or for several days. 1-2-day-old treated (control) males were mated individually (2♀♀:1♂) to virgin sc^{S1} In S B w^a sc^8 (for genetic symbols see ref.[8]). Recessive lethals were tested for in the the F_2. A breeding schema consisting of 3 broods of 3 days duration each was set up, so that treated mature sperm (brood I), spermatids (and sperm) in



Dosage-mortality relationships to oxydemetonmethyl for males of the Hikone R strain (HR) and the Berlin wild strain (Bw).

[1] H. MARTIN, *Pesticide Manual* (British Crop Protection Council, 1968), p. 495.
[2] G. MOHN, Mutation Res., *20*, 7 (1973).
[3] R. FAHRIG, Naturwissenschaften *60*, 50 (1973).
[4] H. KIKKAWA, Jap J. Genet. *28*, 171 (1953).
[5] M. OGAKI and M. TSUKAMOTO, Botyu-Kagaku *23*, 100 (1953).
[6] Z. OGITA, Botyo-Kagaku *23*, 108 (1958).
[7] E. VOGEL, Mutation Res. *11*, 397 (1971).
[8] D. L. LINDSLEY and E. H. GRELL, Carnegie Inst. of Washington Publ. No. 627 (1968).