Metabolism of catecholamines in Mg-deficient, noise-stressed and spontaneously hypertensive rats*)

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Zusammenfassung

Mg-verarmte, lärmgestreßte und spontan-hypertensive Ratten entwickeln eine gesteigerte Empfindlichkeit gegenüber Noradrenalin. Zur Analyse dieses Effekts haben wir die Aktivität der Monoaminooxidase (MAO) und Catecholmethyltransferase (CMT) in der Leber sowie die Bindungseigenschaften der α- und β-Rezeptoren im Herzen dieser Ratten untersucht.

Die Aktivität der MAO und CMT sowie die Affinität und Zahl der α- und β-Rezeptoren zeigten keine signifikanten Unterschiede. Die erhöhte Noradrenalin-Empfindlichkeit ist deswegen wahrscheinlich dadurch bedingt, daß die Catecholamin-induzierbare Desensibilisierung der β-Rezeptoren gestört ist.

Summary

Mg-deficient, noise-stressed and spontaneously hypertensive rats develop an enhanced sensitivity to injected noradrenaline. To analyse this effect, the activity of monoamine oxidase (MAO) and catechol methyltransferase (CMT) in the liver and α- and β-receptor binding in the heart of these rats were determined.

The activity of MAO and CMT showed no significant differences in all groups. Affinity and number of binding sites of α- and β-receptors were also unchanged. Therefore, the enhanced sensitivity to noradrenaline may be caused by a failure of the catecholamine-induced desensitation of β-receptors.

Résumé

Les rats avec déficit magnésique, avec le stress du bruit et avec une hypertension spontanée manifestent une sensibilité accrue à la noradrénaline injectée. Pour analyser cet effet, nous avons étudié l'activité de la mono amine oxydase (M.A.O.) et celle de la catécholméthyltransférase (C.M.T.) dans le foie ainsi que les propriétés de fixation des récepteurs α et β dans le cœur de ces rats.

L'activité de la M.A.O. et celle de la C.M.T., ainsi que l'affinité et le nombre des récepteurs α et β n'ont pas présenté de différences significatives. La sensibilité accrue à la noradrénaline est liée par suite vraisemblablement au fait que la désensibilisation des β-récepteurs induite par les catécholamines est perturbée.

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In Mg-deficient rats, the excretion of adrenaline, and to an even higher degree, noradrenaline is increased especially when the rats are stressed [6]. In Mg-deficient and noise-stressed rats blood pressure is elevated (in preparation) and the hypertensive reaction to injected noradrenaline is increased [9]. To analyse this effect, we measured the activity of MAO and CMT in the liver and the number and affinity of α- and β -adrenergic receptors in the heart of Mg-deficient, noise-stressed rats. For comparison age-matched spontaneously hypertensive rats showing an increased stress [7] and noradrenaline sensitivity [3] were employed.

Materials and Methods

24 male Wistar rats, weighing 120 g were fed ad libitum with a Mg-deficient diet (Altromin C 1035, Mg^{2+} content: 5 mmol/kg, Ca^{2+} content: 180 mmol/kg) for 12 weeks. 12 rats received distilled water with 4 mmol/l $MgCl_2$ ad libitum (groups I, II) and 12 rats received distilled water ad libitum (groups III, IV). One half of the rats (groups II, IV) were noise-stressed with Leq = 95 dB, randomly applied between 8 p.m. and 8 a.m. for 8 weeks, and between 4 p.m. and 8 a.m. for 4 weeks (Leq = energy equivalent continous noise level). 6 control rats and 6 SHR (Okamoto) received a diet rich in Mg (Mg^{2+} content: 80 mmol/kg, Ca^{2+} content: 180 mmol/kg). The hearts and livers were removed under ether anesthesia, rinsed with ice-cold 0.9 % Na Cl and stored at −20° C. The hearts and livers were homogenized at 0° C. MAO activity of liver cell homogenates was determined by the radiometric assay described by *Wurtman* and *Axelrod* [18]. After incubation of (^{14}C)-tryptamine with liver cell homogenate the deaminated radioactive product, (^{14}C)-indolacetic acid was extracted with toluol and counted in a liquid scintillation counter.

CMT activity was determined by the method of *Quiram* and *Weinshilboum* [12] in the 100 000 g supernatant fraction of liver cell homogenate. 3.4-dihydroxybenzoic acid (DBA) was used as a

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substrate for the reaction and (^{14}C)-methyl-5-adenosyl-1-methionine (SAM) was used as the methyl donor. DBA was converted to actively (^{14}C)-labelled 4-hydroxy-3-methoxy-benzoic acid (vanillic acid) by CMT and the radioactive product was separated by organic solvent extraction prior to measurement of the radioactivity in a liquid scintillation counter.

α- and β-adrenoceptors were determined with a 30 000 g fraction of the rat heart homogenate by the method of *Williams* and *Lefkowitz* [17], using (^{3}H)-dihydroergocryptine as a specific antagonist of α-adrenoceptors and (^{3}H)-dihydroalprenolol as that of β-adrenoceptors. Specific binding was defined as the radioactivity bound to membranes which could be displaced by 10^{-5} M phentolamine for α-adrenoceptors and by 10^{-5} M(±) propranolol for β-adrenoceptors. All experiments were performed twice in duplicate-samples.

Results and Discussion

As shown in Table 1, there were no significant differences in the activity of MAO and CMT in the livers of all groups. For Mg-deficient and noise-stressed rats we did not find corresponding values in literature.

For SHR, CMT activity in the hearts was reported to be higher than in normal rats depending on the age of SHR [2]. However, CMT in liver is about 100 times more active than CMT in the heart [5]. Therefore, elimination of injected noradrenaline by CMT in the heart can be neglected.

Also the number and affinity of α- and β-adrenergic receptors in the hearts of all groups were unchanged. The reported number of α-receptors in rat hearts varied between 41 fmol/mg prot. [17] and 307 fmol/mg prot. [1] and the affinity (K_D) varied between 0.4 nmol/l [14] and 11.3 nmol/l [15].

For β-receptors 31.8 fmol/mg prot. [11] and 164 fmol/mg prot. [8, 13] and for K_D values of 1.4 to 3.9 nmol/l [8, 11, 13] were reported. The values found in the present study were within this range (Tab. 1). However, there are also discrepancies in the behaviour of adrenergic receptors in the hearts of SHR. In SHR, a decrease in the number of β-receptors (from 88 to 58 fmol/mg prot.) with unchanged affinity [10] and no alterations in number and affinity of β-receptors were found [11].

From our results we can conclude that the number and affinity for α- and β-receptor binding sites in the heart and the elimination rate of catecholamines is unchanged in SHR and Mg-deficient and noise-stressed rats.

Moreover, the affinity of membrane-bound α- and β-receptors is reduced with decreasing Mg^{2+} concentration in the test [4, 16]. With some α-agonists this effect of Mg^{2+} is abolished in presence of 150 mmol/l Na^{+} [4]. Therefore, at the reduced extracellular Mg^{2+} concentration in Mg deficiency, noradrenaline receptor binding and thus noradrenaline-sensitivity may be reduced. However, noradrenaline-sensitivity was increased in Mg deficiency and stress [6]. Therefore, it is

Tab. 1: Activity of monoamine oxidase [MAO] in liver homogenate, catechol methyltransferase [CMT] in liver cytosol number and affinity of α- and β-adrenergic receptors in the heart of normal, Mg-deficient, noise-stressed and spontaneously hypertensive [SHR] rats. I: mild Mg-deficient unstressed rats, II mild Mg-deficient noise-stressed rats, III: stronger Mg-deficient unstressed rats, IV: stronger Mg-deficient noise-stressed rats. Mean ± SEM of 6 rats.

	Control	I	II	III	IV	SHR
MAO nmol/mg prot x h	66.0 ± 4.2	57.5 ± 2.4	57.5 ± 1.8	56.9 ± 3.0	57.0 ± 2.4	68.4 ± 4.2
CMT µmol/mg prot x h	0.43 ± 0.04	0.42 ± 0.03	0.52 ± 0.02	0.43 ± 0.02	0.41 ± 0.04	0.39 ± 0.03
α-adren. receptors fmol/mg prot	216 ± 21	210 ± 48	204 ± 27	205 ± 31	203 ± 33	215 ± 28
K_D nmol/l	2.7 ± 0.5	2.8 ± 0.3	2.7 ± 0.4	2.6 ± 0.5	2.4 ± 0.4	2.7 ± 0.3
β-adren. receptors fmol/mg prot	216 ± 24	215 ± 29	220 ± 44	213 ± 22	235 ± 38	239 ± 25
K_D nmol/l	2.6 ± 0.4	2.6 ± 0.4	2.5 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	2.7 ± 0.4

suggested to explain the increased noradrenaline sensitivity by a failure of the normally occurring desensitization of catecholamine receptors, leading to a relatively higher number of binding sites in the presence of a high catecholamine concentration.

References

[1] *Ciaraldi, T., Marinetti, G. V.*, Biochem. Biophys. Res. Commun. **74** (1977) 984 – 991.

[2] *Creveling, C. R., Dalgard, N. H., Nikodijevic, B. V.:* Fed. Proc. **28** (1969) 416.

[3] *Dietz, R., Schömig, H., Mann, J. F. E., Rascher, W., Lüth, J. B., Grünherz, N.:* F. Cross. Circ. Res. **43** (1978) I-98-I-106.

[4] *Glossmann, H., Hornung, R., Presek, P.:* J. Cardiovasc. Pharmacol. **2** [Suppl. 3] (1980) 303 – 324.

[5] *Guldberg, H. C., Marsden, C. A.:* Pharmacol. Rev. **27** (1975) 135 – 206.

[6] *Günther, T., Ising, H., Merker, H. J.:* J. Clin. Chem. Clin. Biochem. **16** (1978) 293 – 297.

[7] *Hallbäck, M.:* Acta physiol. Scnad. [Suppl.] **424** (1975 5 – 59.

[8] *Harden, T. K., Wolfe, B. B., Molinoff, P. B.:* Molex. Pharmacol. **12** (1976) 1 – 15.

[9] *Ising, H., Günther, T., Handrock, M., Michalak, R., Schwarze, J., Vormann, J., Wüster, G.-A.:* This symposium.

[10] *Limas, C., Limas, C. J.:* Biochem. Biophys. Res. Commun, **83** (1978) 710 – 714.

[11] *Mukherjee, A., Greham, R. M., Sagalowsky, A. I., Pettinger, W., Mc.Coy, K. E.:* J. Molex. Cell Cardiol., **12** (1980) 1263—1272.

[12] *Quiram, D. R., Weinshilboum, R. M.:* J. Neurochem. **27** (1976) 1197 – 1203.

[13] *Sabouralt, D., Bauche, F., Guidelli, J., Nordmann, J., Nordmann, R.:* Experientia **37** (1981) 227 – 229.

[14] *Sharma, V. K., Banerjee, S. P.:* J. Biol. Chem. **253** (1978) 5277 – 5279.

[15] *Story, D. D., Briley, M. S., Langer, S. Z.:* Eur. J. Pharmacol. **57** (1979) 423 – 426.

[16] *Williams, L. T., Mullikin, D., Lefkowitz, R. J.:* J. Biol. Chem. **253** (1978) 2984 – 2989.

[17] *Williams, R. S., Lefkowitz, R. J.:* Circ. Res. **43** (1978) 721 – 727.

[18] *Wurtman, R. J., Axelrod, J.:* Biochem. Pharmacol. **12** (1963) 1439 – 1441.

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Accelerated aging of rats by Mg deficiency and noise stress*)

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Zusammenfassung

Der Hydroxyprolingehalt als Maß für den Kollagengehalt steigt im Ventrikel von normalen Ratten während eines Alters von 3 bis 30 Monaten linear an.

Die normale Zunahme des Hydroxyprolingehaltes wird durch Mg-Mangel, Lärmstreß und in stärkerem Maße beim Zusammenwirken von Mg-Mangel und Lärmstreß beschleunigt.

Der Hydroxyprolingehalt nimmt bei konstanter Versuchszeit von 12 Wochen mit steigendem Lärmpegel zu.

Summary

Hydroxyproline as a measure of collagen content in the ventricles of normal rats is linearly increased during the age of 3 up to 30 months.

The normal increase in hydroxyproline is accelerated by Mg deficiency, by noise-stress and to a higher degree by the combination of Mg deficiency and noise-stress.

The hydroxyproline content at a constant experimental period of 12 weeks is increased when the noise-level is increased.

Résumé

L'hydroxyproline en tant que mesure de la teneur en collagène dans les ventricules des rats normaux s'accroit linéairement au cours de la période d'âge de 3 à 30 mois.

L'accroissement normal de la teneur en hydroxyproline est accéléré par le déficit magnésique, par le stress du bruit et dans une plus large mesure, par l'action concomitante du déficit magnésique et du stress du bruit.

La teneur en hydroxyproline s'accroit pour une durée d'essai constante de 12 semaines avec un niveau croissant de bruit.

*) Results presented at the 3rd International Symposium on Magnesium, Baden-Baden, 22.—28. 8. 1981.