Magnesium and Nicotinergic Effects on Catecholamine Release from Chromaffin Cells

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Zusammenfassung

Ein *in vitro* Modell, die perifundierte Vena cardinalis posterior des Aals, sezerniert drei Katecholamine. Norepinephrin und Epinephrin werden von dem Äquivalent des Nebennierenmarks sezerniert, während Dopamine wahrscheinlich von einer Komponente der Gefäßwand stammt. In niedriger Konzentration (250 ng/ml) stimuliert Nikotin nur die Sekretion von Norepinephrine, in höherer Konzentration (1 µg/ml) jedoch die Sekretion aller drei Katecholamine. Eine erhöhte Magnesiumkonzentration (5.6 mM) reduziert die Abgabe von NE und E, hat aber keine statistisch signifikante Wirkung auf die DA Sekretion. Nikotin anulliert den Magnesiumeffekt. Die unterschiedliche Reaktion der drei Katecholamine auf eine erhöhte Magnesiumkonzentration könnte möglicherweise zum Anti-Streßeffekt des Magnesiums beitragen.

Summary

In an *in vitro* model, the perifused posterior cardinal vein of the eel, norepinephrine (NE) and epinephrine (E) are released from the equivalent of the adrenal medulla, while dopamine (DA) probably derives from vascular tissue. A low concentration of nicotine (250 ng/ml) stimulates the release of NE, while a higher concentration (1 µg/ml) stimulates the release of all three catecholamines. Magnesium (5.6 mM) reduces NE and E release without a significant effect on DA release. Nicotine overrides the magnesium effect. The differential catecholamine responses to magnesium may contribute to the anti-stress effect of magnesium seen in vivo.

Résumé

In vitro, la veine cardinale posterieur de l'anguille sécréte trois catécholamines. Norepinephrine (NE) et epinephrine (E) derivent des cellules chromaffines, tandis que dopamine (DA) derive probablement de tissu vasculaire. Nicotine, en concentration bas (250 ng/ml) stimule la sécrétion de NE. En concentration elevé (1 µg/ml), nicotine stimule la secretion de les trois catécholamines. Magnésium (5.6 mM) attenue la sécrétion de NE et E sans une effet significant sur DA. Nicotine surmene l'effet de magnésium. Une réponse differentiel des catécholamines a magnésium pourrait augmenter l'effet antistress de magnésium observé in vivo.

Introduction

The anti-stress effect of magnesium is at least in part due to a reduction of catecholamine (CA) release [9, 11]. Since the chromaffin cells of the adrenal medulla are innervated by cholinergic fibers [12], the impact of magnesium could be due to an action on the chromaffin cells, the innervation of these cells, or both. The present studies aimed to clarify the interaction between the cholinergic (nicotinergic) stimulation and magnesium in the control of CA release. We used an animal model, the *in vitro* perifused posterior cardinal vein of the American eel (Anguilla rostrata). In this preparation [3, 4], norepinephrine (NE) and epinephrine (E) are released from chromaffin cells, i.e., the equivalent of the adrenal medulla, whereas dopamine (DA) is probably secreted by an as yet unidentified component of the vascular wall [8].

Material and Methods

The anterior region of the posterior cardinal vein of 43 eels was used. In the eel, the bulk of the chromaffin cells is located directly underneath the endothelium [5, 6] and thus easily reached by the perifusion medium. Details of the perifusion method have been given previously [3]. The perifusion medium ("buffer") was made up as follows: 7.6 g NaCl, 0.22 g KCl, 0.12 g $MgSO_4$, 0.15 g $CaCl_2$, 0.014g NaH_2PO_4, 0.36 g glucose and 2.38 g HEPES in 1000 ml of dist. water. The total duration of the perifusion was 47.5 minutes. The sample taken after 20 minutes was used as the baseline (defined as 100%), and after a subsequent 5 min interval 10 further samples were taken 2.5 min apart. All values were individually normalized as percentages of the baseline value. The average of the 10 normalized values was used for the evaluation of differences between the different experimental groups (Table 1). In groups 1–4, the prerun was 20 min of buffer solution. In groups 5–7, the prerun was 20 min of buffer with increased magnesium concentration. The CAs were determined radioenzymatically, using the CAT-A-KIT (Amersham). Their concentration in the perifusates used for baseline levels varied strongly; probably, this reflects in the case of NE and E the individually varying distribution pattern of the chromaffin cells [5] along the posterior cardinal vein (DA: 40–960 pg/ml; NE: 1.3–6.7 ng/ml; E 3.0–24.4 ng/ml). These values were

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Tab. 1. The Effects of Nicotin and Magnesium on Catecholamine Release from the Posterior Cardinal Vein of the Eel *in vitro*.

		Dopamine	Norepinephr.	Epinephrine
1. Buffer Controls	(N=6)	91±10	84±2	85±2
2. Buffer & 250 ng/ml nicotine	(N=9)	93± 4	92±3*	79±4
3. Buffer & 1 μg/ml nicotine	(N=5)	198± 7***	190± 8***	172±6***
4. Buffer & 10 × magnesium	(N=6)	75±13	80±6	76±4
5. 10 × magnesium throughout	(N=6)	80± 4	67±4***	69±3**
6. 10 × magnesium & 250 ng/ml nicotine	(N=5)	70± 8	71±4	72±3
7. 10 × magnesium & 1 μg/ml nicotine	(N=6)	129±12*	140±8***	145±7***

Note the stimulatory effect of nicotine (Groups 2 and 3 vs. Group 1), the inhibitory effect of magnesium (Group 1 vs. Group 6), and the overriding effect of the higher concentration of nicotine (Group 7). All calculations (Two-tailed T-test for independent measurements) are based on individually normalized values. The data are expressed as Mean ± SEM. *, ** and *** indicate $P < 0.05$, < 0.01 and 0.001, respectively, when compared with buffer controls (Group 1). The P values for Group 7 (10x magnesium & 1 μg nicotin) are the same ones when this group is compared with buffer controls (group 1) and magnesium controls (Group 5). There was no difference between group 6 (Magnesium & 250 ng/ml nicotine) and the pertinent controls (group 5); for clarity, an irrelevant significant difference between Groups 1 and 6 is not indicated.

well within the sensitivity of the assay: DA 15-20 pg, NE and E 2-5 pg per 50 μl sample.

Results

The data are summarized in Table 1. A low concentration of nicotine (250 ng/ml) stimulated the release of NE only (group 2), whereas a higher concentration (1 μg/ml) stimulated the release of all three CAs (group 3). Compared with the control group, the differences for group 3 are 118%, 126% and 102% for DA, NE and E, respectively; compared with the baseline values (= 100%) within group 3, the increases are 98%, 90% and 72%, respectively. A tenfold increased magnesium concentration had no effect on CA release (group 4). However, when the preparation was perifused throughout the experiment with the same magnesium concentration (i.e., 20 min prerun plus 27.5 experimental time), it reduced the release of NE and E (20% and 19% vs. controls; 33% and 31% vs. baseline values, respectively), but had no significant effect on DA (group 5). The low concentration of nicotine (250 ng/ml) did not counteract the magnesium effect (group 6), while the higher nicotine concentration (1 μg/ml) overrode the magnesium effect completely, causing a statistically significant increase of the release of all three CAs (group 7).

Discussion

The present data confirm that (a) the release of all three CAs is stimulated *via* cholinergic, nicotinergic receptors, while (b) NE and E release is reduced by an increased magnesium concentration. Furthermore, they show that NE release is more sensitive to nicotinergic stimulation than E release. The latter observation is compatible with the existence of two different types of chromaffin cells that release NE and E, respectively [5, 13]. At this point, a detailed discussion of this finding is not warranted since it is likely that *in vivo* the action of acetylcholine is modulated by co-released peptidergic secretion(s) [13], and possibly also by muscarinergic receptors. On the other hand, it remains to be seen whether the nicotine-induced increase of DA release is due to a direct impact of the drug, or mediated by E and NE. The macrovessels of both eel and rat release DA from tissue(s) yet to be identified, possibly the endothelium [8, 10]. Probably, this is also the case in the posterior cardinal vein (used in the present studies), and explains the dichotomy between DA release on one hand, and NE and E release on the other, consistently seen under baseline conditions [3, 4]. In contrast, increased concentrations of NE, and particularly of E stimulate DA release [1]. Thus, in the present study, NE and/or E of chromaffin cell origin may have stimulated the release of vascular DA. Since within the physiological range DA causes vasodilation [7], it may act as a "gentle brake" of the vasoconstrictive actions of the other two CAs [2].

The lack of a significant effect of magnesium on DA release in the presence of a drop of NE and E is compatible with a different, i.e., vascular origin of DA [8]. Obviously such dichotomous release favors an impact of DA. Should this finding be confirmed for the *in vivo* situation, it could mean that "magnesium-insensitive" DA release enhances the direct antistress effect which magnesium exerts *via* a reduced NE and E secretion. While this situation may prevail under chronic stress, the effect of nicotine (groups 6 and 7; Table 1) suggests that a strong acute (neural) stimulus may override the magnesium impact.

Acknowledgments

These studies were supported by NSF grant IBN-9408427 to *A. Epple* and a stipend of the Austrian Ministry for Science to *U. Weiss*.

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