Physiol. Res. 53: 357-363, 2004

Hypotensive Effect of Agmatine, Arginine Metabolite, is Affected by NO Synthase

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Received June 9, 2003
Accepted September 29, 2003

Summary

The metabolites of arginine were recently shown to be involved in cardiovascular control. The study addresses the general cardiovascular response of anaesthetized rats to agmatine, a decarboxylated arginine. The relation between two arginine metabolic pathways governed by arginine decarboxylase and nitric oxide synthase was investigated. Intravenous administration of agmatine 30 and 60 μM/0.1 ml saline elicited remarkable hypotension of 42.6±4.6 and 70.9±6.5 mm Hg, respectively. The hypotension was characterized by long duration with half-time of return 171.6±2.9 and 229.2±3.8 s, respectively. The time of total blood pressure (BP) recovery was about 10 min. Dose-dependent relaxation to agmatine was also found in aorta rings *in vitro*. Both doses of agmatine administered 60-180 min after NO synthase inhibition (L-NAME 40 mg/kg i.v.) caused greater hypotension 59.0±7.6 and 95.8±8.8 mm Hg ($P<0.01$ both) compared to animals with intact NO synthase, but this was accompanied by a significant shortening of the half-time of BP return. If agmatine was administered to hypertensive NO-deficient rats (treated with 40 mg/kg/day L-NAME for 4 weeks), similar significant enhancement of hypotension was observed at both agmatine doses, again with a significant shortening of half-time of BP return. It can be summarized that the long-lasting hypotension elicited by agmatine was amplified after acute or chronic NO synthase inhibition, indicating a feedback relation between the two metabolic pathways of arginine.

Key words
Agmatine • Hypotension • NO synthase • NO-deficient hypertension • Aorta relaxation

Introduction

Metabolism of arginine has attracted considerable interest in recent decades as it has been demonstrated that intermediate and/or end-products of individual metabolic pathways of arginine are relevant for the function and control of the cardiovascular system (Ignarro *et al.* 1987, Amezcua *et al.* 1989, Gerová *et al.* 1998). Moreover, its metabolites also participate in structuring the pattern of the cardiovascular system (Garg and Hassid 1989, Nakaki *et al.* 1990, Sládek *et al.* 1996, Kristek and Gerová 1996).

Besides the well known Krebs-Henseleit pathway *arginine → ornithine + urea* mediated by arginase (Krebs and Henseleit 1932), and for the more recently known pathway *arginine → citrulline + NO* mediated by NO synthase (Palmer *et al.* 1988), the last decade has provided the evidence that arginine

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decarboxylase, originally known to operate in plants, bacteria and invertebrates (Wu and Morris 1973), was also found in mammals (Raasch *et al.* 1995, Lortie *et al.* 1996). Arginine decarboxylase governs the metabolic pathway *arginine* → *agmatine*. Agmatine, a biogenic amine, already reported by Kossel (1910), has been recently shown to be involved in the control of vascular smooth muscle tone (Gao *et al.* 1995, Sun *et al.* 1995).

The above three enzyme systems competing for the common substrate arginine were supposed to interact mutually. As far as the metabolic pathway governed by arginase and NO synthase is concerned, a negative feedback has already been demonstrated. The first metabolic product – hydroxy L-arginine – inhibits the activity of arginase (Buga *et al.* 1996, Boucher *et al.* 1999).

Sporadic data are available on the relation of the metabolic pathway governed by NO synthase and arginine decarboxylase and, indeed, they are completely controversial. Whereas Augnet *et al.* (1995) reported selective inhibition of inducible NO synthase by agmatine and Galea *et al.* (1996) found that agmatine induced an inhibition of all three isoforms of NO synthase in the brain tissue, vessel wall and macrophages, Blantz *et al.* (2000), on the other hand, described activation of NO synthase in endothelial cells by agmatine. The controversial experimental results stimulated further investigation. The aim of the present study was to acquire information on the general response of the cardiovascular system to agmatine represented by blood pressure of anesthetized animals, and also to describe the response of vascular smooth muscle in isolated vessels. Moreover, the relation between the metabolic pathway governed by arginine decarboxylase and NO-synthase was addressed.

Methods

Wistar-Kyoto male rats were used for the experiments. The animals were housed in individual cages, under 12-h dark-light cycle, constant temperature (22-24 ºC) and free access to pellet food and water. The procedures and experimental protocol used in the study was approved by the Animal Care Committee of the Slovak Academy of Sciences.

Experiment I

The group for acute experiments consisted of 8 male rats, 12-14 weeks of age, weighing 360-380 g. The animals were anesthetized by sodium thiopental (40 mg/kg b.w. intraperitoneally). The right jugular vein was cannulated for administering the drugs. Immediately after cannulation the animal was given heparin (25 IU). The right carotid artery was prepared, cannulated and connected to a Stattham pressure transducer, blood pressure was recorded on a Physioscript Schwarzer. The trachea was opened and cannulated.

The drug administration started after a 15-min period of stabilization. Agmatine (Agmatine sulfate, Sigma-Aldrich, Germany) in two doses of 30 and 60 μM was administered randomly. Each dose was dissolved constantly in 0.1 ml physiological solution and was administered during a 10-s period. Physiological Krebs solution consisted of (mM): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, $CaNa_2$ EDTA 0.03, ascorbic acid 0.55. The period between individual administrations of agmatine was about 20 min, after preliminary experience of the extremely slow return of blood pressure to steady-state values. Thereafter, inhibition of NO synthase was induced by administration of NG-nitro-L-arginine-methyl ester (L-NAME, Sigma-Aldrich, Germany) in a 40 mg/kg b.w. dose. The respective dose of L-NAME was dissolved in 0.3ml saline solution and administered into the jugular vein during a 3-min time period. After stabilization of blood pressure at a novel high level the same doses of agmatine were administered.

Experiment II

The group consisted of 5 males aged 10 weeks weighing 300 g at the beginning of the experiment. The NO synthase inhibition was induced by L-NAME in a dose of 40 mg/kg/day in drinking water. The period of L-NAME administration lasted 4 weeks. BP was measured in these animals non-invasively on the tail artery by the plethysmographic method each week after 5 min adaptation in a warm chamber. The blood pressure was measured 5 times consecutively and the calculated mean value was taken as representative for statistical evaluation. After four weeks of L-NAME administration, the animals were anesthetized as animals in the Experiment I and their jugular vein was cannulated for administering 30 μM and 60 μM agmatine, and their carotid artery for blood pressure recording.

Experiment III

The thoracic aorta of age-matched non-treated rats was used for *in vitro* studies. Aortic rings (3-4 mm in length) were suspended in organ bath and connected to a

force-displacement transducer Sanborn FT 10 to measure isometric tension, as described earlier (Török *et al.* 1993). The organ bath contained a modified Krebs solution at 37 ºC, aerated with 95 % O_2 + 5 %CO_2. Active tension was induced by phenylephrine (1 μM) and administration of drugs started after the active tension had reached a plateau. Concentration responses to agmatine were determined in a cumulative manner. Isometric tension responses to stimulation of NO synthase activity by increasing doses of acetylcholine (1 nM - 10 μM) in the absence or presence (1 mM) of agmatine in the organ bath was monitored.

Statistics

The individual parameters were expressed as means ± S.E.M. Statistical significance was evaluated using the ANOVA test. Values of $P<0.05$ were considered significant.

Results

Experiments I and II

Original records (Fig. 1) show hypotension elicited by 60 μM agmatine (dissolved in 0.1 ml saline and administered intravenously over a 10-s period). Noteworthy is the long-term hypotension and slow return to the previous BP value. This peculiar time course becomes even more conspicuous when compared with the well known hypotensive response to 10 μg acetylcholine (dissolved in 0.1 ml saline and administered intravenously also over a 10-s period) (Fig. 1).

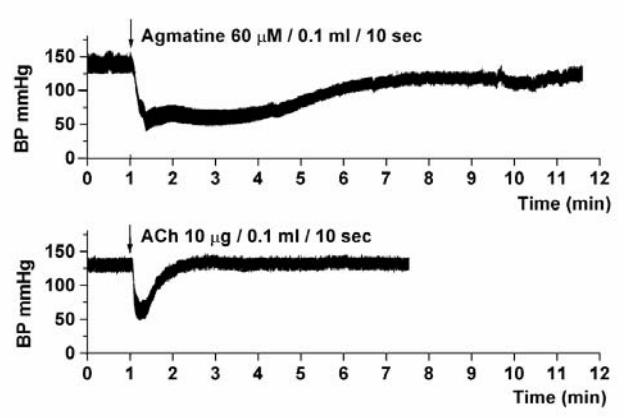

Fig. 1. Blood pressure response to intravenous administration of agmatine (60 μM) and acetylcholine (10 μg)

The mean value of the maximal blood pressure decrease (Exp. I) was 42.6±4.6 mm Hg after the administration of 30 μM agmatine and 70.9±6.5 mm Hg after 60 μM agmatine (Fig. 2).

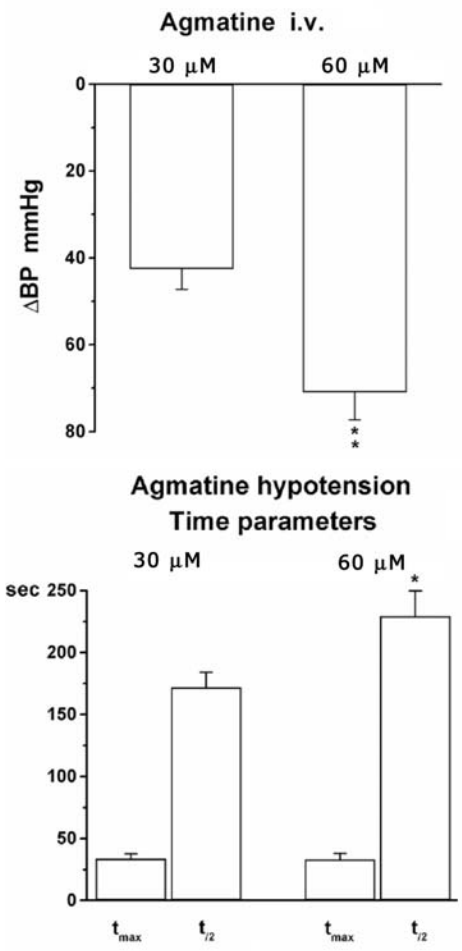

Fig. 2. Blood pressure decrease in mm Hg after intravenous agmatine 30 μM and 60 μM (dissolved in 0.1 ml saline solution, administered in 10 s) (upper panel) and time parameters of hypotension: peak time (t_{max}), half time of BP return ($t/_2$) (lower panel). Mean values ± S.E.M., ** $P<0.01$, * $P<0.05$ significance between two doses.

For a more detailed description of the marked slow return of hypotension to starting values, the following time parameters were estimated: time of peak hypotension was 33.6±0.6 s and 33.0±0.6 s after 30 μM and 60 μM agmatine, respectively. Half-time of return was 171.6±2.9 s and 229.2±3.8 s after 30 μM and 60 μM agmatine, respectively (Fig. 2). The time of a full return of blood pressure to starting values was 563.4±9.4 s and 675.6±11.7 s after 30 μM and 60 μM agmatine, respectively.

The inhibition of NO synthase by L-NAME increased the blood pressure from 131.3±3.7 mm Hg to a stabilized value of 151.5±3.8 mm Hg ($P<0.01$) in a period of 60-180 min (Fig. 3). Agmatine, 30 μM and 60 μM,

administered during the inhibition of NO synthase elicited a blood pressure decrease by 59.0±7.6 mm Hg (P<0.01) and 95.8±8.8 mm Hg (P<0.01), respectively. Both values were significantly augmented in comparison with the corresponding values seen before NO synthase inhibition (Fig. 4).

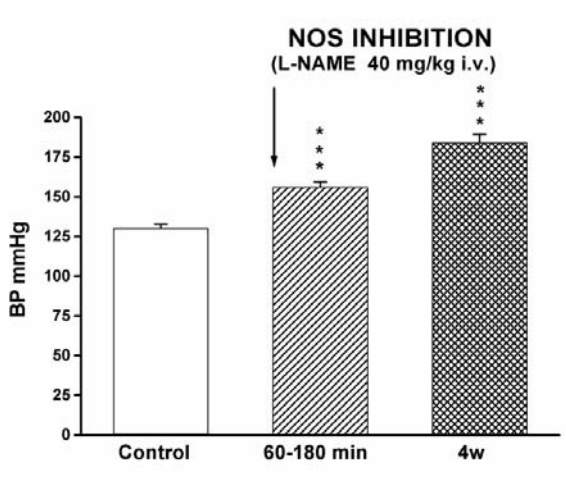

Fig. 3. Steady-state blood pressure values (open columns), BP increase stabilized 60-180 min after the inhibition of NO synthase (L-NAME 40 mg/kg, intravenously, hatched columns), and BP value after NO synthase inhibition lasting 4 weeks (L-NAME (40 mg/kg) administered in drinking water, cross-hatched columns). Mean values ± S.E.M., *** P<0.001

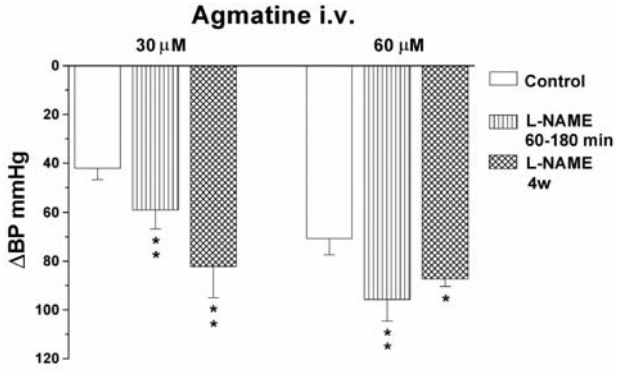

Fig. 4. The extent of BP decrease after intravenous agmatine administration in the dose of 30μM and 60 μM in control animals (open columns), in animals after 60-180 min of NO synthase inhibition (hatched columns), and in animals after 4 weeks´ lasting inhibition of NO synthase (cross-hatched columns). Mean values ± S.E.M. * P<0.05, ** P<0.01

The time parameters of hypotension are presented in Figure 5. Using the two doses, no difference was found in the time to hypotensive peak. Half-time of blood pressure return after 30 μM and 60 μM of agmatine administration was 51.0±10 s (P<0.001) and 58.8±7.8 s (P<0.001), respectively, which is significantly shorter than the corresponding control values of 171.6±2.9 s and 229.2±3.8 s. A similar sequence was found with complete return of blood pressure to starting values: 336.0±42.0 s (P<0.001) and 264.0±35.0 s (P<0.001) vs. 563.4±9.4 s and 675.6±11.7 s after 30 μM and 60 μM agmatine, respectively.

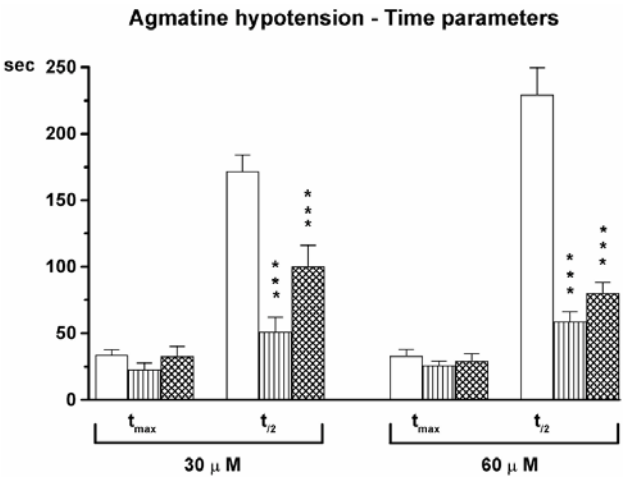

Fig. 5. Time parameters of agmatine hypotension ( ) after intravenous administration of 30 μM and 60 μM doses: peak time (t_{max}), half time of BP return ($t/_2$), controls (open columns), after 60-180 min inhibition of NO synthase (hatched columns) and after 4 weeks´ lasting inhibition of NO synthase (cross-hatched columns). Mean ± S.E.M. *** P<0.001.

Five rats (Exp. II) were administered L-NAME for four weeks; during that period a stabilized hypertension developed. The noninvasive measurement at the beginning of the experiment yielded BP value of 130.0±2.4 mm Hg. Blood pressure increased in the first week, and it was 184.2±5.2 mm Hg (P<0.001) at the end of the fourth week (Fig. 3).

Both doses of agmatine (30 μM and 60 μM) induced a blood pressure decrease of 82.3±12.7 mm Hg (P<0.01) and 87.3±3.1 mm Hg (P<0.05), respectively. These values were significantly enhanced when compared to control values. No significant difference was found in the values of BP decrease between animals subjected to short-lasting and long-lasting NO synthase inhibition (Fig. 4).

The time parameters of agmatine hypotension in hypertensive rats induced by chronic NO synthase inhibition were as follows: peak hypotension was reached at 32.7±7.5 s and 29.2±5.5 s after intravenous administration of 30 μM and 60 μM agmatine, respectively. These values did not differ from control values. The half-time of BP return was 100.0±16.2 s (P<0.001) and 80.0±8.1 s (P<0.001) after 30 μM and

60 μM agmatine, respectively. The values of half-time were significantly shorter compared to the values found in control animals before NO synthase inhibition (Fig. 5). The time of total BP return represented 594.0±23.7 s and 657.0±40.1 s after 30 μM and 60 μM agmatine, respectively. A tendency to shortening of total BP return was seen only after intravenous agmatine administration in the dose of 60 μM.

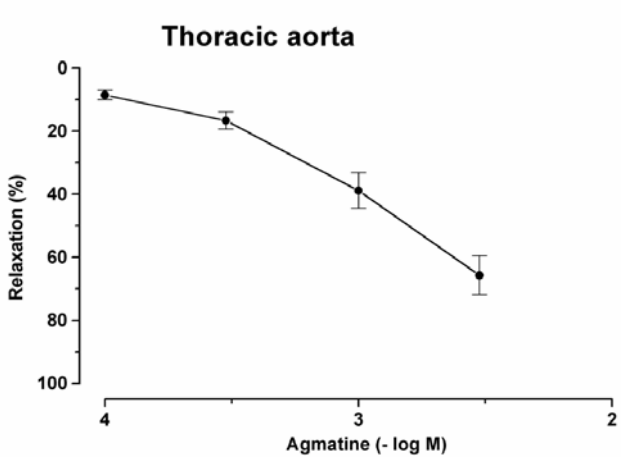

Fig. 6 Concentration-response curve to agmatine in rat thoracic aorta precontracted by phenylephrine (1μM). Values represent the means ± S.E.M. (n=7).

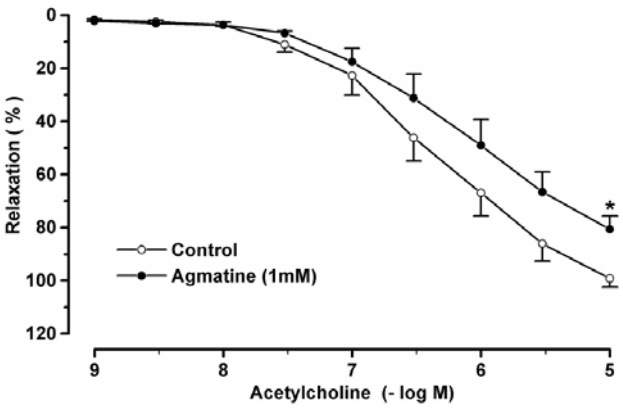

Fig. 7. Concentration-response curve for acetylcholine in the absence (o) or in the presence (•) of 1 mM agmatine. Values represent the means ± S.E.M. (n=6). * P<0.05.

Experimental group III

In the concentration range of 10 μM to 3 mM, agmatine by itself did not induce any response of the resting rings of rat thoracic aorta. In phenylephrine (1 μM) precontracted aortic rings, agmatine induced a concentration-dependent relaxation (Fig. 6). In order to determine whether agmatine influenced the NO synthase in arteries, we constructed dose-response curves for acetylcholine in the absence and presence of agmatine in the incubation medium. Aortic rings pretreated with agmatine (1 mM) yielded attenuated relaxant responses to acetylcholine in the range of 1 nM to 3 μM, being significant at the concentration of 10 μM only (Fig. 7).

Discussion

Agmatine administered intravenously induced dose-dependent hypotension. The hypotension was characterized by a remarkably long duration. The peak hypotension was achieved in about 30 s, however, the half-time of blood pressure restoration required several minutes (depending on the dose used). The time for complete blood pressure reestablishment to the pre-administration level was about 10 min or more.

The first question was whether the changes of cardiac output do contribute to the hypotension. Raasch *et al.* (2000), and our preliminary studies on the effect of agmatine on the Langendorff model of isolated hearts, indicated that agmatine did not affect the functional parameters of isolated myocardium, so that the changes of smooth muscle tone in resistant arteries must have accounted for the observed hypotension.

Inhibition of NO synthase resulting in compromised NO production, elicited an increase in blood pressure, stabilized in about one hour. Agmatine administered at this level distinctly affected the magnitude of hypotension. The extent of hypotension was dose-dependently increased by tens of mm Hg.

This finding was confirmed in the second group of animals with long-term NO synthase inhibition and consequent sustained high blood pressure. In NO-deficient hypertensive rats, remarkable enhancement of BP decrease was also found after agmatine administration. Using a different experimental approach, Galea *et al.* (1996) found an inhibition of NO synthase by agmatine. Our *in vitro* experiments on the thoracic aorta also confirmed the inhibition of NO synthase by agmatine.

Considering the data of the present experiments *in vivo* and *in vitro* and the data provided by Galea *et al.* (1996), it appears that agmatine relaxing effects on vascular smooth muscle cells might fully develop after the reduction of nitric oxide production by NO synthase inhibition. One should hypothesize that NO production by the metabolic pathway *arginine → citrulline* might inhibit the relaxing effect of agmatine on vascular smooth muscle cells. The hypothesis should be extended further: a negative feedback relation between the two metabolic

pathways – one governed by arginine decarboxylase, the other by NO synthase – might be involved.

The results of *in vitro* experiments with thoracic aorta suggest that the mechanisms considered above take place in the vascular wall (most probably in endothelial cells). Whether the idea of a feedback relation between the two above mentioned metabolic pathways of arginine hold true in the whole cascade controlling the vascular smooth muscle tone remains to be further investigated. Sun *et al.* (1995) demonstrated that agmatine had no effect on the rostral ventrolateral medulla and they have suggested that agmatine blockade of transmission in sympathetic ganglia underlies the relaxation of vascular smooth muscle. This idea has been confirmed by Schäfer *et al.* (1999).

The binding of agmatine to specific receptors has not been solved in spite of several trials conducted with α_2-adrenergic receptors or imidazoline I_1 and I_2 receptors (Pinthong *et al.* 1995, Regunathan *et al.* 1996, Török 2001). Irrespective of the presence of agmatine in many organs (Raasch *et al.* 1995), the expression of specific receptors for agmatine as well as the effective influence on the function of respective organs are still waiting for further investigation.

Noteworthy were the time parameters, namely the slow fading of agmatine hypotension. Even more remarkable were the findings that in spite of the marked increase of agmatine hypotension in both acutely and chronically compromised NO production, a distinct increase in the velocity of blood pressure restoration was found. No data dealing with the time course of agmatine hypotension in animals with compromised NO production are available for comparison. A simple explanation of the phenomenon could be proposed, namely the involvement of baroreceptors. Augmented hypotension might induce an amplified baroreceptor reflex by affecting the central sympathetic outflow. This mechanism is conceivable because agmatine has no direct effect on rostral ventrolateral medulla (Sun *et al.* 1995).

The experiments allow to conclude that (i) agmatine induces a distinct hypotension lasting several minutes, and (ii) agmatine-induced hypotension is amplified after several hours of NO synthase inhibition as well as after 4 weeks´ lasting NO synthase inhibition with consequent NO-deficient hypertension. These findings allow to suggest a negative feedback between the two arginine metabolic pathways governed by arginine decarboxylase and NO synthase. Finally, the mechanisms of the increased rate of recovery from the enhanced agmatine hypotension are still not clear and need further experiments.

Acknowledgements

This study was supported by the VEGA grant No. 2/3166/23. Our thanks are due to Anna Buzalková for the reliable assistance in performing the experiments, and Katarína Šoltésová for secretarial work. A preliminary communication (Gerová and Török 2003) was presented at the Meeting of Czech and Slovak Physiological Societies, Plzeň, February 5-7, 2003.

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