Effect of Adrenal Arterial Infusion of Different Mg^{2+} Concentrations on the Spontaneous Release of Catecholamines in the Anesthetized Dog[1] (37248)

H. M. BRECHT, A. HELBER, D. GANTEN, K. HAYDUK, W. NOWACZYNSKI, AND J. GENEST

Clinical Research Institute of Montreal, Montreal, Quebec, Canada

An effect of magnesium ion concentration on the spontaneous release of an "adrenaline-like" substance from the isolated bovine adrenal gland was reported in 1926 by Kusnetzow (1), who found that high concentrations of $MgCl_2$ in the perfusion fluid resulted in an inhibition, whereas low concentrations slightly augmented the liberation of an adrenaline-like substance.

Unphysiologically high Mg^{2+} concentrations have also been proved to suppress the stimulated release of catecholamines from the isolated adrenal gland induced by various substances: acetylcholine, potassium and calcium (2, 3), phenylethylamine, *d*-amphetamine and tyramine (4) and chlorpromazine (5).

The influence of Mg^{2+} on the spontaneous release of norepinephrine (NE) and epinephrine (E) separately by the adrenal gland *in vivo* has not yet been studied. The present study was performed under more physiological conditions than previously employed.

Since higher than normal Mg^{2+} concentrations decrease aldosterone secretion (6), which influences the transformation of NE to E (7, 8), and since angiotensin stimulates catecholamine release from the adrenal medulla (9–13), we investigated also whether the Mg^{2+} effect was mediated through the renin–angiotensin–aldosterone system.

Materials and Methods. Animals. Nine male and female mongrel dogs, weighing 20–30 kg, were kept for one week prior to surgery in individual cages and were fed a mixture of Purina Chow and commercial Rover meat. The adrenal glands of five of them (experimental group) were exposed to elevated Mg^{2+} concentrations, whereas those of the other four dogs (control group) received infusions of physiological Mg^{2+} concentrations.

Surgical procedure. The dogs were anesthetized with sodium pentobarbital (25 mg/kg body weight) and intubated. A polyethylene tube inserted in the femoral artery was connected to a pressure transducer, and blood pressure was continuously recorded with a Grass model 7 Polygraph throughout the experiment (for schematic diagram see Fig. 1). The left phrenicoabdominal vein was carefully dissected (all apparent branches were tied off) and cannulated with a No. 260 silastic tube[2] for collection of adrenal venous blood. A No. 240 polyethylene tube was placed in the left renal artery in such a position that the end of the tube was near to the origin of the adrenal branches of the renal artery. The perfusion fluid was infused through this tube into the arterial blood supply of the left adrenal gland by means of an infusion pump (Sigma Motor model TM 11) at a constant flow rate of 1.5 ml/min. The left kidney was removed to prevent a "flowing-off" of the infusion fluid into the renal circulation. The amount of infusion fluid was too small to have any influence on the adrenal perfusion pressure, which was solely determined by the arterial blood pressure of the animal. The entire surgical procedure was carried out retroperitoneally, the abdominal cavity remaining closed. The nervous as well as the blood supply to the left adrenal gland remained intact.

Infusion fluids. The control infusion fluid was a Krebs–Ringer-bicarbonate (KRB) solution. Solutions of high Mg^{2+} concentra-

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[2] Silastic-medical-grade-tubing, Dow Corning, Medical Products Division.

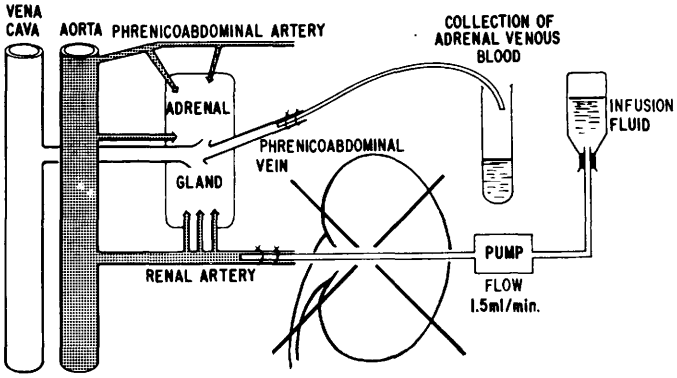

FIG. 1. Surgical technique used for the arterial infusion of the adrenal gland in the anesthetized dog.

tions were prepared by the addition of magnesium sulfate to the KRB solution in concentrations between 10 and 20 mEqiv/l. Although the infusion fluid contained rather high concentrations of Mg^{2+}, its concentration determined in adrenal venous plasma during the course of the experiment was only between control and twice control values in four dogs (*i.e.*, 1.8–3.1 mEqiv/l) and only 5.5 mEqiv/l in a fifth. This dilution effect was due to the mixing of a very small amount of infusion fluid with arterial blood in the renal artery and adrenal gland. We have assumed that the adrenal venous concentration of Mg^{2+} at equilibrium was equivalent to the concentration to which the adrenal medullary cells were exposed. The amounts of magnesium infused during an experiment was less than 5 mEqiv and was not sufficient to alter significantly the magnesium concentration in peripheral plasma as measured at the end of the experiment.

Experimental procedure. Each experiment lasted four hours. After a control infusion period of one hour, the adrenal gland was exposed to the test solutions for three hours. Samples (20 ml) of adrenal venous blood were taken at hourly intervals. Collection periods were less than five minutes. For the remaining 55 min of the hour's periods, the tube inserted in the phrenicoabdominal vein was closed and the adrenal venous blood flowed off totally into the systemic circulation.

Since the phrenicolumbar vein between adrenal gland and vena cava could not be tied off during the collection of blood, only part of the adrenal venous blood was obtained; all results are expressed in concentrations per liter of adrenal venous plasma.

NE and E were determined in plasma by a modification (14) of the trihydroxyindole derivative method of Anton and Sayre (15).

Plasma sodium and potassium were determined by flame emission spectroscopy (Unicam model SP-900 Flame Spectrophotometer), and plasma magnesium and calcium by atomic absorption spectroscopy (Perkin–Elmer model 303 Atomic Absorption Spectrophotometer).

Blood samples for the determination of plasma renin activity (PRA) were taken from the cephalic vein before anesthesia, one hour after the control period, and at the end of the experiment. Plasma renin activity was measured according to the method of Boucher (16) as modified by Granger (17).

Results. Blood pressure. The blood pressure remained stable in both groups of dogs during the whole experimental period.

Cations in adrenal venous blood. Plasma concentrations of Ca^{2+}, K^+, and Na^+ in adrenal venous blood did not change significantly during the infusion with control and with high Mg^{2+} solutions. Thus, changes in the Mg^{2+} concentration of the adrenal blood supply had no effect on the concentrations of the other cations in the adrenal venous blood.

Release of norepinephrine and epinephrine from the adrenal gland. (a) Response to control solution. In order to rule out the influence of the stress of surgery and of anesthe-

TABLE I. Release of Catecholamines from the Adrenal Gland During Control Infusion at Physiological Mg^{2+} Concentrations.

Control dog		Hours of infusion 1	2	3	4
	Mg[a]	1.9	1.85	1.6	1.7
1 (F)	NE + E[b]	68.8	64.7	70.3	82.9
	% E	80.2	82.1	79.8	81.8
	Mg	1.6	1.5	1.4	1.6
2 (M)	NE + E	14.6	15.1	14.0	16.0
	% E	76.7	73.5	75.0	75.0
	Mg	1.5	1.6	1.6	1.6
3 (M)	NE + E	9.3	10.4	9.3	8.8
	% E	79.6	82.7	80.6	78.4
	Mg	1.9	1.6	1.5	1.7
4 (F)	NE + E	62.7	46.2	50.3	48.0
	% E	69.5	74.5	67.8	71.2

[a] mEqiv/l Adrenal venous plasma.
[b] μg/l Adrenal venous plasma.

sia, we infused four dogs with the control solution for four hours and measured magnesium and catecholamine concentrations in adrenal venous plasma (Table I). These did not change during the four hours. The total catecholamine concentration showed wide individual variations from dog to dog (8.8–82.9 μg/liter), but the NE/E ratios remained fairly stable in each dog during the 4-hr control infusions.

(*b*) *Response to infusion at high Mg^{2+} concentration.* The average Mg^{2+} concentration in the adrenal venous plasma after one hour of control infusion with normal KRB solution in the group of five dogs was 1.84 ± 0.07 (SEM) mEqiv/l. The adrenal glands were then exposed to different high Mg^{2+} concentrations (Table II). When Mg^{2+} concentration was slightly increased to levels between 1.8 and 2.1 mEqiv/l (Dog 1), the output of both NE and E decreased after the first hour of high Mg^{2+} infusion, with a return to near-control levels at the end of the experiment. A similar effect on the release of catecholamines was seen in Dog 2, in which the Mg^{2+} concentration of the adrenal venous plasma was brought up to levels between 2.3 and 2.4 mEqiv/l. When the adrenal gland was exposed to still higher Mg^{2+} concentrations (2.6–2.8 mEqiv/l (Dog 3), the inhibition of catecholamine release persisted until the end of the infusion. The inhibition of NE and E release was even more marked when Mg^{2+} concentrations were increased to 3.0–3.1 mEqiv/l (Dog 4). One experiment (Dog 5) was done with unphysiologically high Mg^{2+} concentrations (5.2–5.5 mEqiv/l) in the adrenal venous plasma and showed similar results, although the inhibition was not marked.

In these five dogs the percentages of epinephrine found in the adrenal venous plasma after the first hour of control infusion were very similar, being 83.6 ± 1.7 (SEM) % of the total, despite the large individual differences in the total catecholamine concentration. When the Mg^{2+} concentration in the arterial blood flowing through the adrenal gland was increased, the concentration of both NE and E decreased, but within a certain range of Mg^{2+} concentration (2.3–3.1 mEqiv/l), the percentage of epinephrine was somewhat lower (Dog 2–4). This preferential decrease of epinephrine was not noted when the Mg^{2+} concentration in the adrenal

TABLE II. Release of Catecholamines from the Adrenal Gland in the Presence of High Mg^{2+} Concentrations.

Experimental dog		Hours of infusion 1 (Control)	2	3	4
	Mg[a]	1.6	1.8	1.9	2.1
1 (F)	NE + E[b]	47.4	22.8	7.4	38.7
	% E	84.2	91.7	75.7	81.4
	Mg	1.9	2.4	2.3	2.3
2 (F)	NE + E	14.2	6.9	6.5	12.5
	% E	84.5	78.3	67.7	72.0
	Mg	1.9	2.8	2.8	2.6
3 (F)	NE + E	22.4	11.4	7.4	4.4
	% E	88.4	79.8	78.4	72.7
	Mg	1.8	3.1	3.0	3.0
4 (F)	NE + E	18.5	5.0	1.6	1.9
	% E	81.1	58.0	68.7	68.4
	Mg	1.9	5.2	5.3	5.5
5 (F)	NE + E	21.9	9.4	10.2	6.0
	% E	79.9	75.5	83.3	76.7

[a] mEqiv/l Adrenal venous plasma.
[b] μg/l Adrenal venous plasma.

venous plasma was either only slightly elevated up to 2.1 mEqiv/l (Dog 1) or unphysiologically increased to 5.2, 5.3, 5.5 mEqiv/l, respectively (Dog 5).

Plasma renin activity (PRA) in peripheral venous plasma. Plasma renin activity in peripheral blood was increased 2-fold after one hour of control infusion as compared to the plasma renin activity before anesthesia. This rise in plasma renin activity was most probably due to the anesthesia and the surgical procedure as previously demonstrated by our group. The further three hour's infusion produced no change of PRA, either in the control or in the experimental groups.

Discussion. The influence of divalent cations on the release of catecholamines from the isolated adrenal gland has been the subject of many investigations during the past years. The conclusions from these studies have nevertheless been ambiguous and contradictory.

In the isolated adrenal gland, Mg^{2+} inhibited the stimulated release of catecholamines most probably by its competition with calcium, the essential link in stimulus–secretion coupling at the adrenal medulla (2, 4). Kusnetzow (1) described two contradictory effects of Mg^{2+} on the spontaneous release of an adrenal-like substance from the isolated adrenal gland (see "Introduction"). When the isolated medullary granules were examined, increased Mg^{2+} concentration proved not to be an effective inhibitor of either the spontaneous or the evoked release of catecholamines (18); on the contrary, Mg^{2+} accelerated the catecholamine release from isolated granules (19). In our *in vivo* experiments in which the adrenal gland was anatomically intact, normally innervated, and supplied with arterial blood, increasing concentrations of Mg^{2+} resulted in a graded inhibition of the spontaneous catecholamine output. A slight elevation of Mg^{2+} concentration by 15–20% above control was already sufficient to inhibit the spontaneous release of NE and E for about two hours, after which time an escape phenomenon was observed. When higher Mg^{2+} concentrations (up to double the control) were employed, the inhibition lasted for the entire infusion period (3 hr). Thus, the duration of the inhibition seemed to depend on the Mg^{2+} concentration.

In all cases in which Mg^{2+} concentration was higher than normal, catecholamines in adrenal venous plasma were found to be decreased. There are at least three possible mechanisms whereby Mg^{2+} could be involved. (a) Synthesis of catecholamines might be suppressed. (b) Release of catecholamines might be blocked. (c) Uptake of catecholamines by the granules might be accelerated. The catecholamine content of the total adrenal gland before and after exposure to high Mg^{2+} concentrations was not measured. But the last possibility is likely, since there is much evidence that Mg^{2+} plus ATP stimulates the uptake process of catecholamines in the adrenal granules (20, 21). An increased uptake of catecholamines in the adrenal granules when exposed to high Mg^{2+} concentrations might be a possible mode of action of the inhibited release during resting conditions of the adrenal gland.

The role of calcium in the stimulus–secretion coupling is well established, but since it has been demonstrated that calcium has no effect on the spontaneous release of adrenaline from the adrenal gland (2, 5) and since there was no change of the calcium concentration in the adrenal venous plasma, the effect of Mg^{2+} must be mediated otherwise.

Another interesting finding was the preferential decrease of epinephrine found in the adrenal venous plasma when the adrenal gland was exposed to concentrations of Mg^{2+} of 2.3 to 3.1 mEqiv/l. This observation might be explained in the following way. The enzyme that mediates the conversion of NE to E in the adrenal medulla, phenylethanolamine *N*-methyl-transferase (PNMT), is controlled by ACTH and adrenocortical hormones (7, 8, 22, 23). Of the corticoid hormones also aldosterone has been demonstrated to increase the synthesis and activity of this enzyme (8). When we couple this fact with the conclusion of Helber *et al.* (6) that a 30–50% increase of the Mg^{2+} concentration in the blood supplying the dog adrenal gland significantly inhibited the aldosterone secretion, we might assume that a marked depression of aldosterone secretion caused by Mg^{2+} was associated with a decreased activity of the enzyme PN MT, which in turn led to a relatively lower

epinephrine concentration in the adrenal venous plasma.

Plasma renin activity was unchanged in peripheral venous plasma during the adrenal arterial infusion with physiological or with high Mg^{2+} concentrations. Thus, alterations in catecholamine release from the adrenal medulla did not affect PRA; conversely, changes in PRA, due to the surgical and experimental procedure, could not have influenced the release of catecholamines.

Summary. The effect of magnesium ion concentration on the spontaneous release of catecholamines from the adrenal gland has been studied in anesthetized dogs under nearly physiological conditions. A very slight elevation (15–20%) of Mg^{2+} concentration in the blood supply of the adrenal gland was found to be sufficient to inhibit the spontaneous release of norepinephrine and epinephrine. Higher concentrations of Mg^{2+} (up to double the control) had still greater effects, but in this case a preferential decrease of epinephrine in the adrenal outflow resulted. A possible involvement of aldosterone in the differential release of norepinephrine and epinephrine is discussed. Plasma renin activity in peripheral blood was not affected and the effect of Mg^{2+} does not seem to be mediated through the renin–angiotensin system.

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