Influence of Magnesium and Cysteine on Vasopressin-Induced Contractions in Various Canine Blood Vessels

Evidence has accrued to suggest that magnesium (Mg^{++}) ions selectively 1. increase the affinity of neurohypophyseal polypeptides (e.g. vasopressin, oxytocin and their synthetic analogues) for their receptors in smooth muscle and 2. potentiate the contractile actions of these hormones on smooth muscle[1]. Various in vitro studies have suggested that potentiation of structurally different neurohypophyseal hormone analogues by Mg^{++} on different effector systems (uterus, mammary gland, blood vessels) is inversely related to potency of the molecules and not to intrinsic differences between uterine smooth muscle, myoepithelial or vascular smooth muscle receptors, per se[2]. Recent work with depolarized uterine smooth muscle[3] and avian pulmonary arteries[4] show, however, that the maximal contractile responses to vasopressin in Mg^{++}-free solution are greatly decreased whereas those elicited by oxytocin under similar conditions are not thus possibly questioning the homogeneity of neurohypophyseal hormone receptor systems. In addition, it has recently been demonstrated on rat myometrium that Mg^{++} cannot only affect the affinity of the hormone for its receptor, but can also alter the maximal contractile response[5].

Furthermore, other in-vitro studies[6], utilizing 7 synthetic analogues of vasopressin, have shown such a varied spectrum of different relative affinities and maximal contractile responses (i.e. intrinsic activities) of the various analogues on 6 different canine blood vessels that it is tempting to speculate that the vasopressin receptor, at least, on different blood vessels within a single mammalian species may not be identical. That is, among the target sites there might be chemical or steric differences within the receptor molecules. Although the experiments in the present report are essentially preliminary, they could be used to support such a concept.

Materials and methods. Various canine blood vessels were obtained from either sex (10–16 kg), cut helically and set up isometrically in vitro essentially similar to that described previously[6]. All vascular strips were equilibrated for 2 h in muscle chambers containing Krebs-Ringer bicarbonate solution, the composition of which has been reported previously[6]. The Krebs-Ringer bicarbonate solution was oxygenated continuously with a 95% O_2 – 5% CO_2 mixture and kept at 37 °C (pH 7.2 to 7.4). Complete, cumulative log dose-response curves, similar to those of the technique of VAN ROSSUM[7], were

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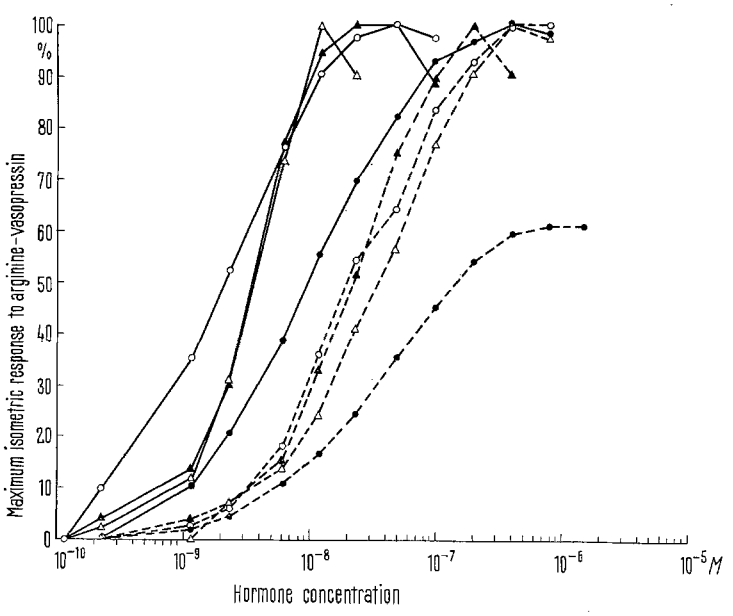

Fig. 1. Cumulative dose-response curves of 8-arginine-vasopressin on in vitro canine blood vessels mounted isometrically in Krebs-Ringer bicarbonate solution without added magnesium and with 1.2 m*M* added magnesium. Abdominal aorta without added Mg^{++} (●---●); abdominal aorta with Mg^{++} (●—●); femoral artery without added Mg^{++} (○---○); femoral artery with Mg^{++} (○—○); renal artery without added Mg^{++} (▲---▲); renal artery with Mg^{++} (▲—▲); carotid artery without added Mg^{++} (△---△); carotid artery with Mg^{++} (△—△). All vascular muscle preparations were exposed to Mg^{++}-free solutions for 1 h prior to determining experimental cumulative dose-response curves. Each curve represents an average of at least 6 experiments with 5 different dogs.

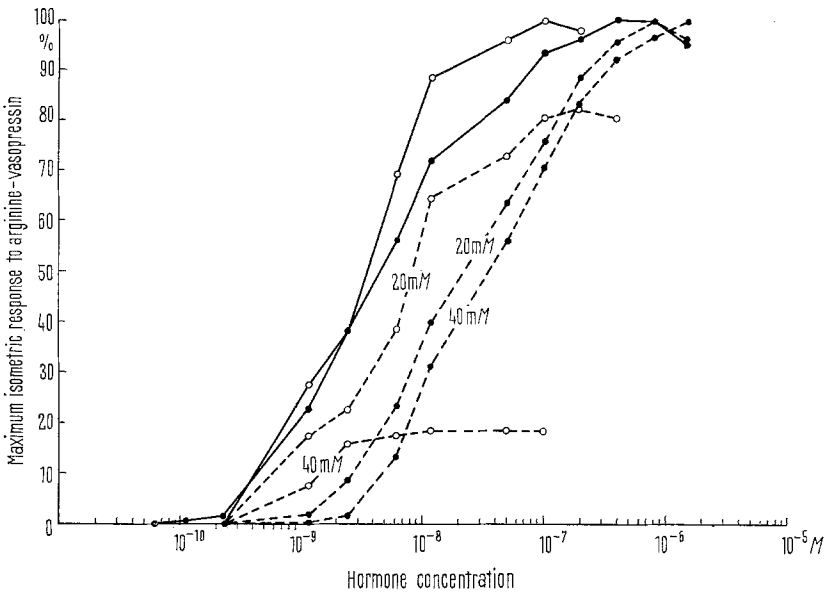

Fig. 2. Cumulative dose-response curves of 8-arginine-vasopressin on in vitro canine femoral arteries and abdominal aortae mounted isometrically in Krebs-Ringer bicarbonate (1.2 m*M*-Mg^{++}) solution without added cysteine HCl and with 20 or 40 m*M* cysteine HCl. Abdominal aorta without added cysteine HCl (●—●); abdominal aorta with 20 and 40 m*M* cysteine HCl (●---●); femoral artery without added cysteine HCl (○—○) femoral artery with 20 and 40 m*M* cysteine HCl (○---○). All experimental vascular strips were exposed to cysteine for 15 min prior to determining cumulative dose-response curves (cysteine was left in the muscle baths during the cumulative dose-response curves). Each curve represents an average of at least 5 experiments with 4 different dogs.

obtained for synthetic 8-arginine-vasopressin on the various canine blood vessels (i.e. abdominal aortae, femoral, renal and carotid arteries). Experiments were not performed on any of the vascular strips until a particular preparation yielded 2 consecutive, complete cumulative dose-responses curves to 8-arginine-vasopressin which were within 10% of one another. Preparations exhibiting tachyphylaxis to vasopressin were discarded.

Results and discussion. If the composition of the vasopressin receptor on different mammalian blood vessels within a single species is indeed homogeneous (i.e. similar) one should expect Mg^{++}-free solutions or contact with certain sulfhydryl compounds (e.g. cysteine) to induce parallel shifts of the dose-response curves to the right with no reduction in maximum response on all blood vessels. (The SOMLYOS[8] have provided rather extensive evidence on certain canine vascular smooth muscle preparations which strongly suggest that Mg^{++} ions increase the affinity of vasopressin for its receptor on blood vessels without altering the maximum response, whereas MARTIN and SCHILD[9], working with isolated rat uterine smooth muscle, have provided evidence that certain sulfhydryl compounds, like cysteine, may compete with neurohypophyseal peptides for the receptor rather specifically.) The present findings with either Mg^{++}-free solutions (Figure 1) or contact of different canine vascular smooth muscle preparations with different concentrations of cysteine (Figure 2) reveal, however, 2 kinds of general phenomena: 1. either a parallel shift of the dose response curves to the right with no reduction in maximal response (i.e. no loss in intrinsic activity) on certain canine vessels (seen in Mg^{++}-free media with renal, femoral and carotid arteries and in the case of contact with cysteine in abdominal aortae), or 2. a rightward shallowing of the dose-response curves together with a marked reduction in maximal response (i.e. loss in intrinsic activity) on other canine vessels, possible indicative of a non-competitive type of inhibition in this latter case (see e.g. abdominal aortae in Mg^{++}-free media and femoral arteries upon contact with cysteine).

Although these preliminary findings do seem to suggest that some sort of difference in the vasopressin receptor may exist in different blood vessels, further work will be required to corroborate this tenet since other explanations for the observed differences, at least in the case of the Mg^{++}-free experiments, could be invoked[8, 10–12].

Zusammenfassung. In-vitro-Versuche erwiesen, dass Magnesium-freie oder Cystein-enthaltende Krebs-Ringerlösung unterschiedlich auf vasopressininduzierte Kontraktionen an verschiedenen Blutgefässen des Hundes wirken. Zwei verschiedene Phänomene wurden beobachtet: entweder eine parallele Verschiebung der Dosis-Wirkungskurven nach rechts ohne Verminderung der maximalen Verkürzung oder eine Neigung der Dosis-Wirkungskurven mit einer auffallenden Verminderung der maximalen Verkürzung. Es wird auf Grund der Befunde angenommen, dass die Vasopressin-Rezeptoren verschiedener Blutgefässe nicht identisch sind.

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