Stimulation of brain mast cells by compound 48/80, a histamine liberator, evokes renin and vasopressin release in dogs

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Matsumoto I, Inoue Y, Shimada T, Matsunaga T, Aikawa T. Stimulation of brain mast cells by compound 48/80, a histamine liberator, evokes renin and vasopressin release in dogs. Am J Physiol Regul Integr Comp Physiol 294: R689–R698, 2008. First published January 9, 2008; doi:10.1152/ajpregu.00453.2007.—Because degranulation of brain mast cells activates adrenocortical secretion (41, 42), we examined whether activation of such cells increases renin and vasopressin (antidiuretic hormone: ADH) secretion. For this, we administered compound 48/80 (C48/80), which liberates histamine from mast cells, to pentobarbital-anesthetized dogs. An infusion of 37.5 μg/kg C48/80 into the cerebral third ventricle evoked increases in plasma renin activity (PRA), and in plasma epinephrine (Epi) and ADH concentrations. Ketotifen (H1 histamine receptor antagonist) significantly reduced the C48/80-induced increases in PRA and Epi, but potentiated the C48/80-induced increase in ADH and elevated the plasma Epi level before and after C48/80 challenge. No significant changes in mean arterial blood pressure, heart rate, concentrations of plasma electrolytes (Na+, K+, and Cl−), or plasma osmolality were observed after C48/80 challenge in dogs with or without SPX. Pyrilamine maleate (H1 histaminergic-receptor agonist) significantly reduced the C48/80-induced increase in PRA when given intracerebroventricularly, but not when given intravenously. In contrast, metiamide (H2 histaminergic-receptor agonist) given intracerebroventricularly significantly potentiated the C48/80-induced PRA increase. A small dose of histamine (5 μg/kg) administered intracerebroventricularly increased PRA twofold and ADH fourfold (vs. their basal level). These results suggest that in dogs, endogenous histamine liberated from brain mast cells may increase renin and Epi secretion (via the sympathetic outflow) and ADH secretion (via the central nervous system). Stimulation of brain mast cells by compound 48/80, a histamine liberator, evokes renin and vasopressin release in dogs.

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interesting hypothesis that when immediate hypersensitivity breaks out either in the CNS or in the periphery, histamine liberated from mast cells may activate the release of various stress hormones. However, it remains unclear whether or not brain mast cells participate in altering renal function in some physiological or pathophysiological conditions. In this study, we set out to determine whether and how activation of brain mast cells might induce renin and ADH secretion.

MATERIALS AND METHODS

Animals. All experimental protocols used in this study conformed to the Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences (approved by Physiological Society of Japan, revised 2002). Adult male mongrel dogs weighing 10.2 to 16.8 kg were used for the experiments. On the day before the experiment, which started at around 9:00 A.M., a 22-gauge stainless-steel tube was implanted as a cerebral guide cannula into the cerebral third ventricle (VIII) of each animal, using a stereotaxic instrument under pentobarbital sodium anesthesia (25 mg/kg iv). The stereotaxic coordinates were 20 mm anterior and 7 mm dorsal to the external aural meatus line, and 0 mm from the midline. After the guide cannula had been firmly anchored to the skull using dental cement, an indwelling catheter for the withdrawal of peripheral blood samples [to be assayed for plasma renin activity (PRA), ADH, and catecholamines] was inserted into the inferior vena cava via a branch of the femoral vein. When required, bilateral splanchnicectomy (SPX; resection of the left and right thoracic splanchnic nerves, which involve the so-called greater and lesser splanchnic nerves; n = 8 dogs) or sham SPX (n = 6) was performed around the adrenal glands. To lessen the effect of surgery on the experimental results, the retroperitoneal route (i.e., without opening the abdomen) was employed for the SPX. The basic method was developed for the taking of blood samples directly from the adrenal vein (56) and was used here with some modifications (42). In sham SPX animals, the same operation was performed as for real SPX, except that no splanchnic nerves were cut. The incisions for the indwelling catheter and the resection of the splanchnic nerves were closed with sutures after administration of penicillin G (30,000 U, intramuscularly) and 2% xylracine jelly (2 ml, given hypodermatically around the incisions; Fujisawa, Japan). The animals were placed in a heated recovery room, with their rectal temperature maintained at above 37.5°C, and allowed to recover for about 24 h. By 8 h after the induction of anesthesia for the above procedures, all animals were awake and could drink water normally of their own volition. Since SPX animals had not undergone a laparotomy operation, total loss of blood and extracellular fluids during the surgery was estimated to be less than 15 ml.

At 9:00 A.M. on the day following the preliminary operation, each animal was reanesthetized with pentobarbital sodium (25 mg/kg iv). Supplementary pentobarbital was given as required to maintain an absent pulpal reflex. Drugs dissolved in artificial cerebrospinal fluid were administered into V III—after the pH had been adjusted (7.4) and the required concentration obtained—at a rate of 15.6 μl/min through a 27-gauge stainless-steel tube fixed into the cerebral guide cannula. Mean arterial blood pressure (MAP) and heart rate (HR) were monitored (via an indwelling catheter in a branch of the femoral artery that had been implanted during the preliminary operation) using a strain-gauge pressure transducer (Statham P23AC) and recorded on a polygraph system (AP620G; Nihon Kohden). Recordings were begun before, and continued after, an intracerebroventricular infusion of C48/80 or histamine. To confirm correct placement of the cerebral cannula, cresylv violet dye was infused into V III (in the same way as the pharmacologic challenge) at the end of the experiment, and dye distribution within the brain was examined in each animal.

Experimental procedure. Drugs were infused into V III using an infusion needle (0.35 mm ID, stainless steel; inserted through the guide cannula) at an infusion rate of 15.6 μl/min for 5 or 10 min. Blood sampling was started at 1300 on the day following the operation. Each venous blood sample (about 3 ml) was delivered into a chilled tube containing heparin at a rate of 2 ml/min. Sampling was done at nine time points: 10 min before, and 5, 10, 20, 30, 40, 60, 90, and 120 min after the start of an intracerebroventricular infusion of either C48/80 (Sigma-Aldrich) or histamine (Sigma-Aldrich). Value of measures (PRA and ADH) at 0 min in figures were substituted by that at 10 min before the start of intracerebroventricular infusion of the corresponding each experiment. Following immediate centrifugation at 4°C, the plasma was removed for storage with EDTA (15%) and then frozen for not more than 7 days, at −80°C until needed for estimation of PRA, catecholamines, and ADH. The blood cells from each sample were resuspended in the same volume of saline and returned to the animal before the next sampling period through the indwelling venous catheter. Such replacements ensured that the hematocrit of all blood samples was within the range 42 to 46%.

When required, an intracerebroventricular injection of pyrilamine maleate, a H₁-histaminergic antagonist (5 μg/kg over 10 min; Sigma-Aldrich), or metiamide, an H₂-histaminergic antagonist, (50–500 μg/kg over 10 min; SKF-Japan) was given, from 5 min before to 5 min after the start of the C48/80 challenge. Ketotifen fumarate (Sigma-Aldrich), a prophylactic antiasthma drug with a mast cell-stabilizing action (22, 39), was given in one of two ways: 1) simultaneous administration [by a 20-min infusion (100–2,000 μg/kg) into the V III (from 15 min before to 5 min after the start of the C48/80 infusion)], or 2) chronic administration [via the oral route (per os, po) every morning for 7 days, ending on the day before the experiment (2 mg/day; a dose comparable to that given to allergic individuals)]. In dogs either pretreated chronically with ketotifen or not given ketotifen at all (i.e., only given 37.5 μg/kg C48/80/5 μg/kg histamine was given subsequently as an intracerebroventricular challenge at 15 min after the start of the C48/80 infusion (i.e., 30 min after the final blood sampling in the experiment on the effect of ketotifen). This was done to establish whether or not ketotifen’s histaminergic effect, one of the side effects of this drug, might be responsible for any change in the C48/80-evoked adrenal cortical secretory response.

Determination. PRA was determined by a modification of the method of Harber et al. (24) using a commercially available ANG-I radioimmunoassay/PRA assay kit (Vackstar). After allowing the frozen sample to thaw in an ice bath, 1.0 ml of plasma sample was incubated for 90 min at 37°C with 2 ml of 0.1 M maleate buffer (pH 6.0), containing 6.0 mM EDTA and 2.0 mM phenyl methane sulfonfluoride in the presence of a 48 h-nephrectomized dog plasma (used as renin substrate). The unknown amount of ANG-I generated during 37°C incubation competed with a fixed amount of [125I]-labeled ANG-I for a fixed amount of anti-ANG-I serum. Both the unknown samples and the standards were assayed in duplicate. The intra-assay and interassay coefficients of variation were 10.3 and 12.2%, respectively. The specific enzyme activity of renin was expressed as nanogram ANG-I formed by mixing 0.5 ml buffer containing either standard or 0.5-ml extract of sample with 100 μl of 125I-labeled ANG-I and cross reaction was 2% with lysine-

Plasma ADH was determined by the method of Camps et al. (9) using a commercially available rabbit anti-serum (Calbiochem-Behring). The buffer used for the radioimmunoassay was barbital (20 mmol/l, pH 8.6) containing 0.14 M NaCl, 0.01 M EDTA, and 10 μl of normal rabbit serum. The hormone was extracted from 0.5 ml of plasma sample using 2 ml of cold 98% ethanol. After evaporation of the ethanol extract in a N2 stream, the radioimmunoassay was performed by mixing 0.5 ml buffer containing either standard or 0.5-ml extract of sample with 100 μl of antisemur. After incubation for 20 h at 4°C, 500 disintegrations per min of [125I]-labeled AVP (100 μl) was added, and tubes were incubated again for 24 h at 4°C. After separation of free and bound hormone using charcoal-dextran, radioactivity was determined. No cross reaction of the antibody was obtained with oxytocin, and cross reaction was 2% with lysine-

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vasopressin. The interassay and intra-assay coefficients of variation were 14.3% and 11.7%, respectively. Recovery was more than 65%, and the sensitivity was 0.3 pg/tube. Plasma catecholamines were measured by the coulometric electrochemical determination method (32), with the minor modification previously reported (43), using a high-performance liquid chromatographic analytic system (ESA: model 5100A).

Statistical analysis. All data are presented as means ± SE. Data were analyzed using a one- or two-way ANOVA, with correction for repeated measures, followed by an appropriate post hoc test (Fisher’s protected least significant difference) for multiple comparisons.

RESULTS

Infusion of C48/80 into the third cerebral ventricle increases PRA. In intact normal dogs, infusion of C48/80 (3.75, 37.5, and 375 µg/kg) via the intracerebroventricular route increased PRA in a dose-dependent manner (Fig. 1A). The peak response was seen at around 30 min after the start of the infusion and when the two higher doses were used (37.5 and 375 µg/kg), PRA remained elevated until 120 min and then gradually declined (but had not returned to the basal level by 150 min after the start of the infusion). In splanchectomized dogs: 1) the basal level of PRA was only 60% of that seen in intact animals, and 2) C48/80-induced increases in PRA were not seen in the first 60 min or so, although PRA showed a significant elevation above the basal level at 90 and 120 min in spite of the resection of the bilateral splanchic nerves (Fig. 1B).

Histamine liberator stimulates renin secretion. To examine whether or not the C48/80-induced increase in PRA depends on mast cells, we examined the effect of pretreatment with ketotifen, a mast cell-stabilizing drug (Fig. 2A). Although ketotifen has a weak anti-H1-histaminergic action (22, 39), as well as a stabilizing effect on mast cells, no significant suppressive effect on the C48/80-induced increase in PRA was seen when ketotifen (2,000 µg/kg) was coadministered with 37.5 µg/kg C48/80 via the intracerebroventricular route. However, a complete prevention of the C48/80-induced increase in PRA was observed in dogs that had been given ketotifen orally (2 mg/day) for 1 wk before the experiment (Fig. 2A). An intracerebroventricular administration of a large dose of ketotifen (2,000 µg/kg) plus vehicle did not significantly alter plasma PRA. We next examined whether the anti-H1-histaminergic action of ketotifen, a side effect of this agent, might contribute to the above attenuation of the C48/80-evoked renal response. To this end, 5 µg/kg histamine (minimum effective dose) was given via the intracerebroventricular route at 30 min after the final blood sampling (at 150 min after the start of the C48/80-challenge) in dogs either chronically pretreated with ketotifen or not given ketotifen at all. In such animals, the PRA (measured at 30 min after the start of the histamine infusion) was significantly increased vs. the basal level, with the increase in ketotifen-pretreated dogs not being significantly smaller than that in ketotifen-untreated dogs (Fig. 2B).

A small dose of histamine given into VIII evokes renin release. To examine whether histamine given intracerebroventricularly evokes a release of renin, a small dose of histamine was given into VIII in intact animals. An intracerebroventricular administration of 5 µg/kg histamine, the minimum effective dose for stimulating the HPA axis in dogs (42), evoked an increase in PRA with a rapid onset and rapid recovery (Fig. 3). Indeed, a significant increase in PRA was detected at 5 min after the administration of histamine; the level then continued to rise and peaked at about 30 min before declining back to the basal level. The magnitude of the increase in the initial phase (5–40 min) was comparable to that induced by 37.5 µg/kg C48/80, whereas in the late phase (60–120 min), the PRA level was significantly lower (at the 90- and 120-min time points) than that induced by 37.5 µg/kg C48/80. Neither MAP nor HR altered significantly after an intracerebroventricular infusion of 5 µg/kg histamine (relevant data are shown in Ref. 42). When 5 µg/kg histamine was given via the intravenous route, an increase in PRA was not seen (Fig. 3).

H1-histaminergic antagonist attenuates, and H2-histaminergic antagonist potentiates, the C48/80-induced increase in PRA. To examine whether histamine liberated from brain mast cells participates in the C48/80-induced increase in PRA, we used H1- and H2-histaminergic antagonists. The C48/80-induced increase in PRA was completely prevented by pretreatment with pyrilamine maleate, an H1 blocker, when it was
administered to V1H (Fig. 4A) but not when it was administered intravenously at the same dose (data not shown). There was no significant change in PRA after an infusion of pyrilamine plus vehicle to V1H. In contrast, pretreatment with metiamide, an H₂ blocker, via the intracerebroventricular route significantly potentiated the C48/80-induced increase in PRA (from 40 to 120 min after the pharmacological challenge), but pretreatment with metiamide had no effect when it was given intravenously at the same dose (data not shown). No significant change in PRA was observed when metiamide plus vehicle was given via the intracerebroventricular route (Fig. 4B). This lack of effect of each histaminergic antagonist (anti-H₁ or anti-H₂) when given via the peripheral route on the C48/80-induced increase in PRA would indicate that histamine liberated from brain mast cells acts within the CNS itself.

Effects of intracerebroventricular C48/80 on cardiovascular function and humoral factors in the plasma. As cardiovascular dysfunction and plasma factors (osmolality or electrolytes) are thought to modulate the renin-angiotensin system, we measured plasma osmolality and electrolytes, as well as MAP and HR, to see whether or not they changed after the pharmacological challenge (Table 1). No significant change in MAP or HR was detected after an administration of C48/80, and the plasma levels of electrolytes (Na⁺, K⁺, and Cl⁻) and plasma osmolality were within their normal physiological ranges both before and after C48/80 administration.

Effects of intracerebroventricular C48/80 on plasma catecholamines. Plasma catecholamines are thought to act as renin secretagogues. To examine whether the plasma levels of catecholamines might be related to the C48/80-evoked increase in PRA, we measured plasma Epi and norepinephrine (NE) before and after administration of vehicle or C48/80 in intact animals with or without ketotifen pretreatment and in animals with SPX (Table 2). The plasma Epi level was significantly increased at 5, 10, and 20 min after the start of an infusion of C48/80 in intact animals. In intact dogs pretreated with ketotifen, such C48/80-induced increases in Epi were not observed. In animals with SPX, the basal level of plasma Epi was one-half that seen in intact animals, and its level was unchanged at all sampling points after an infusion of C48/80. In intact animals, the plasma NE level was within the physiological range both before and after the C48/80 challenge. In animals pretreated with ketotifen, the plasma NE level remained within the physiological range throughout the study period [although at 30–120 min, it tended (nonsignificantly) to be higher than in intact animals], and there were no significant differences between before and after the pharmacological challenge. In dogs with SPX, the plasma NE levels before the
and plasma NE levels was found both in the early period (Fig. 5A; $r = 0.72$) and in the late period (Fig. 5B; $r = 0.68$). However, although both PRA and Epi increased significantly in response to the C48/80 challenge, a significant correlation between PRA and Epi was not observed either in the early period (Fig. 5A) or in the late period (Fig. 5B). In dogs with SPX, the plasma Epi concentration stayed at a very low level (22–28 pg/ml) throughout the entire sampling period (before and after the C48/80 challenge), while the PRA values covered a wide range (1.8–11.5 ng ANG-I·ml⁻¹·h⁻¹). On the other hand, the plasma NE level in the SPX group covered a wide range and reached high values (range, 280–648 pg/ml). Nevertheless, our analyses of the early phase (Fig. 5C) and the late phase (Fig. 5D) failed to reveal a high coefficient of correlation between PRA and plasma NE levels in SPX dogs.

**Increases in plasma ADH in response to C48/80 infusion (and effects of ketotifen pretreatment and SPX).** An intracerebroventricular infusion of C48/80 (37.5 μg/kg) increased the plasma ADH concentration significantly at 5, 10, and 20 min after the challenge (Fig. 6A). This C48/80-induced increase in ADH was transient (return to basal level at 30–40 min after the challenge). Thereafter, the ADH concentration tended to increase progressively until the end of the study period at 120 min (although a similar tendency was also seen in the vehicle group). In dogs given ketotifen (2 mg/day) via the oral route for 7 days before the C48/80 challenge, the transient C48/80-induced increase in ADH was completely prevented, but not the late tendency to increase (which seemed to be independent of the C48/80 challenge, as mentioned above) (Fig. 6B). When a 37.5 μg/kg C48/80 challenge was delivered in animals with SPX, the transient C48/80-evoked increase in ADH was greatly potentiated at 10 and 20 min, and the level then returned to basal values at 30 min (as in intact animals) (Fig. 6C). In the late phase (in which there was a nonsignificant increase in both intact and ketotifen-treated animals), the ADH level in SPX animals was elevated significantly at 90 and 120 min after the challenge.

**A small dose of histamine given into the V III evokes ADH release.** To examine whether histamine given intracerebroventricularly induces a release of ADH, a small dose of histamine was given into the V III in intact animals. Such intracerebroventricular administration of 5 μg/kg histamine evoked an increase in plasma ADH, as well as in PRA (Fig. 7A). The plasma ADH concentration was increased significantly at 5 min after the histamine administration and it peaked at about 20 min, then recovered quickly (return to basal level by 40 min). Comparing the PRA (or ADH) response induced by intracerebroventricular histamine (5 μg/kg) with that induced by intracerebroventricular C48/80 (37.5 μg/kg) revealed the following difference between these stimulants. Although the peak PRA level reached after histamine administration (at 30 min) was 0.9 times that induced by C48/80, the peak ADH level (at 20 min) induced by histamine was 2 times greater than that induced by C48/80 (Fig. 7B).

**DISCUSSION**

Although brain mast cells are known to be highly concentrated in the hypothalamus, especially in the median eminence (ME) (38, 41, 42, 53), their precise physiological role remains...
Effects of C48/80 on humoral factors and cardiovascular indexes

80-induced increases in PRA, ADH, and Epi. Although keto-
secretion and significant increases in plasma ADH and Epi.
of both renin (5, 30, 33, 44, 55) and ADH (2, 7, 15, 57)
central histamine that is known to participate in the regulation
examine whether brain mast cells might be the source of the
increase in adrenocortical secretion induced by C48/80
did not lead to any significant suppression of the C48/80-
Table 2. Effects of pretreatment with ketotifen or SPX on plasma levels of catecholamines

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unclear. Because C48/80 is a mast cell-degranulating agent
(42, 46, 52), its administration into the Vh might be expected
to evoke physiological responses initiated or regulated by the
chemical mediators liberated from brain mast cells. Indeed, we
previously showed in dogs that activation of mast cells located
in the ME stimulates the HPA axis via histamine released
within the CNS, either in response to an antigenic challenge
follow ing passive sensitization by IgE (41) or in response to
C48/80 given into the Vh (42). Here, in a study designed to
examine whether brain mast cells might be the source of the
central histamine that is known to participate in the regulation
of both renin (5, 30, 33, 44, 55) and ADH (2, 7, 15, 57)
secretion, we first showed that an intrace r o b r a n c h i a l
administration of C48/80 evokes a marked increase in renin
secretion and significant increases in plasma ADH and Epi.

Pretreatment with ketotifen significantly reduced these C48/
80-induced increases in PRA, ADH, and Epi. Although keto-
tifen has a weak anti-H1-histaminergic action in addition to its
antiallergic actions (22, 39), simultaneous administration of a
large dose of ketotifen (2,000 µg/kg icv) together with C48/80
did not lead to any significant suppression of the C48/80-
induced increases in PRA. Furthermore, in our previous study,
such coadministration of ketotifen with C48/80 did not affect
the increase in adenocortical secretion induced by C48/80
given into the Vh in intact dogs (42). When 5 µg/kg histamine,
the minimum effective dose eliciting adenocortical secretion
(42, 55) was given intrace r o b r a n c h i a l y at 30 min after the
final blood sampling in dogs chronically pretreated with keto-
tifen (Fig. 2B), PRA was increased significantly from the basal
level at 30 min after the start of the histamine infusion. This
increase was not significantly smaller than that seen in animals
given C48/80 without chronic ketotifen pretreatment (Fig. 2B).

This seems to indicate that in the present experimental proto-
col, ketotifen has only a very weak anti-H1-histaminergic action
(22, 39), and instead, it acts mainly as a mast-cell
stabilizer. In addition, the C48/80-induced increase in PRA
was suppressed when a small dose of pyrilamine, an H1-
receptor antagonist, was coinfused into the VIII, but not when
it was given intravenously. In contrast, pretreatment with
metiamide, an H2-receptor antagonist, augmented the C48/80-
induced increase in PRA. We reported before that an H1-
receptor antagonist attenuates and an H2-receptor antagonist
potentiates an HPA response induced by C48/80 (42) without
any accompanying significant changes in either the plasma
histamine concentration (42) or cardiovascular indexes (42).
Consequently, these data suggest 1) that brain mast cells
induce increases not only in PRA, but also in ADH and Epi via
histamine liberated from brain mast cells induces renin
secretion via histaminergic receptors located within the brain itself.

Further, the C48/80-induced increases in PRA, ADH, and
Epi are unlikely to be secondary to changes in MAP, HR, or
plasma factors (namely, osmolality or the electrolytes Na+
, K+
, and Cl−
) since such changes were not observed in the
present study. In rats, infusion of histamine (3.8–60 µg) into
the lateral cerebral ventricle reportedly increases PRA in a
dose-dependent manner, while prior intrace r o b r a n c h i a l
infusion of an H2-receptor antagonist abolishes the increase in
PRA induced by restraint stress (44). Thus, there may be a
species difference (rat vs. dog) in the central histaminergic
receptor-subtype mediating the regulation of renin secretion.
Because ketotifen suppressed the C48/80-induced increases in
PRA, ADH, and Epi, even when the drug was given via the
oral route, the mast cells responding to the C48/80 infused into

Table 2. Effects of pretreatment with ketotifen or SPX on plasma levels of catecholamines

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<tr>
<th>Variable</th>
<th>Treatment</th>
<th>−10</th>
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<td>Epi, pg/ml</td>
<td>Intact (Vehicle)</td>
<td>39.0±7.05</td>
<td>58.2±9.3</td>
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<td>47.5±6.8</td>
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<td>47.3±5.90</td>
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<td>184±16.8</td>
<td>95±16.0</td>
<td>64.8±6.9</td>
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<td>SPX + C48/80</td>
<td>64.3±15.8</td>
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<td>42±16.6</td>
<td>34.0±20.3</td>
<td>49.4±13.4</td>
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<td>NE, pg/ml</td>
<td>Intact (Vehicle)</td>
<td>201±21.9</td>
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<td>247±26.3</td>
<td>263±39.7</td>
<td>273±30.1</td>
<td>275±23.4</td>
<td>260±10.7</td>
<td>271±28.3</td>
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<td>Ketotifen + C48/80</td>
<td>231±27.1</td>
<td>246.5±41.7</td>
<td>220±32.5</td>
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</table>

Plasma epinephrine (Epi) and norepinephrine (NE) concentrations before and after an administration of vehicle in intact dogs (n = 6) or C48/80 in intact animals (n = 8), in animals chronically pretreated with ketotifen via the oral route for 7 days (n = 7), or in animals with splanchicectomy (n = 6). Values are means ± SE. *P < 0.05 vs. intact animals given vehicle; †P < 0.05 vs. intact animals given C48/80 alone; ‡P < 0.05 versus before value (−10 min, basal level) within the same group.
the V_{III} must inhabit brain site(s) not protected by the blood-brain barrier (BBB). The ME is the most likely candidate because: 1) it lacks the BBB, 2) it contains a high concentration of mast cells (38, 41, 42, 53), 3) such mast cells can be sensitized passively by IgE given via the peripheral route (41), and 4) many degranulated mast cells were found in ME after a C48/80 challenge (42).

Various lines of evidence indicate that the renal sympathetic nerves exert an important influence over renin secretion via both α- (1, 13, 14, 51) and β-adrenergic receptors (1, 3, 14, 26, 31, 35, 48) in the kidney. Because an infusion of C48/80 into the V_{III} evokes an increase in plasma Epi via the adrenomedullary glands (42), we expected such an elevation of Epi to increase in both Epi and PRA seen in the early phase after C48/80 challenge (42).

Although both PRA and NE increased significantly in the late phase in SPX animals, these increases seem to be independent of each other [no significant correlation between PRA and NE being found either in the early phase (Fig. 5C, r = 0.07) or in the late phase (Fig. 5D, r = 0.16)]. Thus, our data indicate that 1) the thoracic splanchnic nerves (including the so-called major and minor splanchnic nerves) contain fibers that mediate central histamine-induced renin secretion, 2) such renin secretion may be dependent on neither plasma Epi nor plasma NE, at least in the present experimental protocol, and 3) the sympathetic activation induced by C48/80 is restricted to the visceral organs governed by the thoracic splanchnic nerves and does not involve the cardiovascular system. If the plasma level of NE reflects a significant correlation between either plasma NE (r = 0.16) or plasma Epi (r = 0.02) by linear regression analysis.

Fig. 5. Correlations between plasma levels of catecholamines (NE or Epi) and PRA during early or late period after a C48/80 challenge (37.5 μg/kg) in intact (A and B) or splanchnicectomized (C and D) animals. A: PRA values in early period [from −10 min to 40 min (6 time points in each individual animal)] after an intracerebroventricular C48/80 challenge plotted against plasma level of NE (bold symbols) or Epi (light symbols) [at the same time points] in individual intact animals (n = 8). PRA values show a high correlation with plasma NE (r = 0.72) but not with plasma Epi (r = 0.19), by linear regression analysis. B: PRA values in late period [from 60 min to 150 min (4 time-points in each individual animal)] after an intracerebroventricular C48/80 challenge plotted against plasma level of NE (bold symbols) or Epi (light symbols) [at the same time points] in individual intact animals (n = 8). PRA values show a high correlation with plasma NE (r = 0.68) but not with plasma Epi (r = 0.13), by linear regression analysis. C: PRA values in early period (from −10 min to 40 min after the C48/80 challenge) plotted against plasma level of NE (bold symbols) or Epi (light symbols) in individual SPX animals (n = 5). Data are for the same time points as in Fig. 5A. PRA values show no correlation with either plasma NE (r = 0.01) or plasma Epi (r = 0.06) by linear regression analysis. D: PRA values in late period (from 60 min to 150 min after the C48/80 challenge) plotted against plasma level of NE (bold symbols) or Epi (light symbols) in SPX animals (n = 5). Data are for the same time points as in Fig. 5B. PRA values show no correlation with either plasma NE (r = 0.16) or plasma Epi (r = 0.02) by linear regression analysis.
A macula densa of the kidney (14, 48). Surprisingly, SPX animals displayed a late-phase increase in PRA despite their lack of intact nerves driving renin release. One explanation might lie in the existing evidence that the control of renin secretion involves nonneuronal mechanisms under many physiological conditions (14). Another possibility is that the elevation of PRA was mediated by fibers in the first lumbar splanchnic nerves distributing to the aorticorenal ganglion (since, to avoid noxious effects on the kidney, we did not resect those nerves). In any event, the late elevation in PRA would appear to be due, at least in part, to mechanisms additional to those controlled by the central histamine-thoracic splanchnic nervous outflow.

In view of the prevention of the C48/80-induced increases in PRA and plasma Epi by SPX, it was surprising that SPX seemed to augment not only the C48/80-induced increase in ADH, but also the late-phase increase in plasma NE. Such potentiation of the increases in ADH and NE might be due to the cardiovascular-regulating system showing compensatory or hypersensitive responses as a result of a lack of afferent inflow from the kidneys. Such enhancements in ADH and plasma NE responses have been seen in spaceflight (45) and in hindlimb-unloaded animals (49, 64). Indeed, mechano- and chemosensitive information transmitted from the kidneys to the paraventricular nucleus (PVN) of the hypothalamus via the renal afferent nerves has been shown to contribute to the modulation of ADH release (14, 58, 60).

**Fig. 6.** Effect of C48/80 on plasma antidiuretic hormone (ADH) concentration, together with effect of an antiallergic agent or splanchnicectomy on the C48/80-evoked increase in ADH. A: effect of C48/80 on plasma ADH concentration in animals given either vehicle (○, n = 5) or 37.5 µg/kg C48/80 (●, n = 8) into VIII. B: effect of ketotifen on the C48/80-evoked ADH in intact animals. C48/80 was given into VIII, with or without ketotifen (2 mg/day) being given chronically via the oral route for 7 days, ending on the day before the C48/80 challenge (●, n = 7). C: effect of SPX on the C48/80-evoked increase in ADH. 37.5 µg/kg C48/80 was challenged via the intracerebroventricular route in dogs with splanchnicectomy (●, n = 5) or in intact dogs (○, n = 8). SPX significantly potentiated the C48/80-induced effect on ADH secretion. Values are means ± SE. *P < 0.05 vs. vehicle control; #P < 0.05 vs. intact animals given C48/80 alone.

Fig. 7. Effect of histamine on plasma level of ADH. A: comparison between increases in plasma ADH induced by 5 µg/kg histamine (●, n = 7) and 37.5 µg/kg C48/80 (○, n = 7), each given via the intracerebroventricular route in intact dogs. B: comparison of peak increases in PRA (left) and ADH (right) at 30 and 20 min, respectively, after a histamine or C48/80 challenge. Values are means ± SE. *P < 0.05 vs. vehicle control; #P < 0.05 vs. animals given C48/80.
In this study, not only a small dose of histamine, but also C48/80, evoked a significant increase in plasma ADH. It is well known that histamine given into the cerebral ventricle greatly elevates ADH secretion (2, 7, 15, 57, 63). When we administered 37.5 μg/kg C48/80 into the V₃, the increase in ADH from the basal level (2.6 times) was roughly equal to that in PRA (2.3 times). However, when 5 μg/kg histamine was administered to the V₃, the ADH increase (4.2 times) was larger than the increase in PRA (2.5 times). These data may show that histamine given into the V₃ can diffuse easily into brain areas around the V₃, such as the PVN of the hypothalamus. Indeed, the histamine liberated from mast cells located in ME (in response to C48/80 given into the V₃) would not spread so easily and would be unlikely to reach such distant brain areas (61). Another explanation might be that there are differences in sensitivity between the histamine receptors in the hypothalamic PVN responsible for increasing ADH secretion and those responsible for increasing renin secretion.

Mast cell-dependent immediate hypersensitivity reactions, such as anaphylactic shock (28, 46), are frequently associated with peripheral cardiovascular dysfunction (10, 12, 18, 65). In the view of the increases in renin and ADH secretions evoked by central mast cells, previous studies have shown that the concentration of histamine in the brain increases during hemorrhage (30) or dehydration (33) and that central histamine participates in hemorrhage-induced renin secretion (29, 30, 44). Furthermore, manipulations that increase the central histamine content [e.g., an administration either of histamine itself (29) or of a histamine N-methyltransferase inhibitor (30) into the V₃] rescue animals from critical hemorrhagic hypotension by increasing MAP and HR via an activation of the renin-angiotensin system (30) and may be via release of ADH, too. There is growing recognition that activation of the HPA axis by central mast cells may contribute to the suppression of the immediate hypersensitivity reactions and subsequent inflammation induced by peripheral mast cells (23, 41) since glucocorticoids can reduce the severity of the cardiovascular dysfunction (34, 47, 50) and suppress the immune response (11, 47, 50). This would indicate a possible physiological importance of the increases in PRA and ADH evoked via brain mast cells. Indeed, the brain mast cell-dependent secretion of vasoactive substances (such as renin and ADH) could potentially help to save the life of an individual by ameliorating the effects of the cardiovascular dysfunction that would otherwise occur during an outbreak of type-I allergy.

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REFERENCES

R698  BRAIN MAST CELLS ACTIVATE RENIN AND ADH RELEASE