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Footshock-induced rise of rat blood histamine depends upon the activation of postganglionic sympathetic neurons

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Abstract

We have previously shown the existence of a novel peripheral reflex inhibitorily modulating the vas deferens sympathetic activity. An interaction between noradrenergic and histamine-containing neurons is involved in this reflex. As an overall mechanism of sympathetic autoregulation, we found that enhanced sympathetic activity in the rat during the stress induced by brief inescapable footshocks caused a marked rise of blood histamine that was seemingly dependent upon sympathetic activity. This rise was prevented by either previous ganglionic blockade with hexamethonium or chronic guanethidine-induced sympathectomy. Previous adrenal demedullation did not impair this rise. Thus, it appears that only the sympathetic postganglionic neuron, interacting with a histamine-containing neuron, is involved in the rise of blood histamine induced by footshocks. © 1998 Elsevier Science B.V.

Keywords: Noradrenergic neuron; Histamine; Peripheral; Neuronal; Sympathetic autoregulation; Blood; Stress

1. Introduction

The discovery of histamine-containing neurons in the central nervous system (Schwartz et al., 1980) has stimulated the search for similar neurons in the periphery. Histamine-containing neurons have been shown in sympathetic ganglia (Panula et al., 1985) and histamine-containing neuronal pathways have been detected in the gut (Häppöla et al., 1985). Our previous work has shown the presence of crossed histamine-containing neuronal pathways at the level of the sympathetic ganglionic clusters of the vas deferens (Campos, 1983, 1988; Campos and Domínguez, 1995). This histamine-containing neuronal system is involved in a peripheral short-loop reflex, in which noradrenergic neurons interact with contralateral sympathetic ganglionic histamine-containing neurons (Campos and Domínguez, 1995). The latter cause a contralateral reciprocal inhibitory modulation of vas deferens sympathetic activity (Campos and Briceño, 1992). Similar inhibitory influences arising from the contralateral stellate ganglion on the sympathetic activity of the dog heart enhanced by unilateral cardiac sympathetic nerve stimulation have been shown (Campos and Briceño, 1992). An interaction of this sort might be involved in a general mechanism of peripheral sympathetic autoregulation. If this is so, enhancement of sympathetic activity during footshock-stress in the awake rat should lead to increases in blood-histamine levels, probably neuronal in origin, as a result of the stimulation of peripheral histamine-containing neurons. In agreement with this point of view, we report here that increases in rat blood histamine, due to brief inescapable footshock-stress, are dependent upon the activation of postganglionic sympathetic neurons. This finding lies within the context of a peripheral interaction between noradrenergic and histamine-containing neurons involved in a reflex that modulates sympathetic activity.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 200–300 g, were used. The rats were housed in the experimental laboratory room at least one week before the experiments were performed. The rats were fasted 24 h prior to the experi-

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ment and were allowed to drink only a 10% sucrose solution at libitum.

2.2. Footshock experiments

Footshock experiments were performed in an adjoining room to prevent the control rats from perceiving the stress induced in the other rats. The stress consisted of mild inescapable electric shocks (15 Hz, 5 ms, 70 V, over a 5-min period) applied to the foot pads of the freely moving rat, through a stainless steel wire grid at the bottom of a plexiglass cage (23 cm × 10 cm × 13 cm). The grid was connected in parallel to an electronic stimulator (Grass S-48). During footshocks, the rats showed vocalization, piloerection, defecation and urination, but were apparently normal at the end of the brief stress period.

2.3. Blood sampling

Immediately after footshocks were stopped, the rats were anesthetized with sodium pentobarbital, 80 mg/kg i.p. After surgical opening of the abdominal cavity, a 5-ml blood sample was drawn from the inferior vena cava, by means of a chilled plastic syringe containing 0.1 ml 5% sodium EDTA in saline solution. The same procedure for blood sampling was used in control rats not receiving footshocks.

2.4. Determination of hematocrit and leukocyte count

Before addition of 1 M $HClO_4$ to precipitate proteins in the blood samples, 100 μl of blood was removed to determine hematocrit and leukocyte count in a Coulter counter S-Plus JR (Hialeah, FL, USA).

2.5. Fluorometric analysis of amines

When [^{3}H]histamine is injected into the blood circulation of Sprague–Dawley rats, the amine is rapidly taken up into cellular elements and its rate of disappearance from plasma is much faster than from whole blood (Johnson et al., 1966). Therefore, we thought it was safer to investigate changes in histamine levels in rat whole blood during a brief enhancement of sympathetic activity.

The blood sample drawn was immediately transferred to a chilled 12-ml plastic centrifuge tube, and 5 ml 1 M $HClO_4$ was added to the tube and mixed thoroughly with the blood. Protein precipitation was completed by centrifugation in the cold at 12 000 rpm for 15 min. A 6-ml volume of supernatant was used for the fluorometric determination of histamine, according to Schwartz et al. (1970), with modifications at the elution step from the cellulose column: a priming 1.5-ml 0.4-M NaCl aliquot was added to the column and discarded. No histamine was lost at this step, as monitored with tritiated histamine or fluorescence reading. Elution was then carried out with 2 ml 0.2 M NaCl, and the entire eluate was used to measure histamine fluorescence by condensation of the amine with *o*-phthalaldehyde in a strong alkaline medium. Recoveries of histamine (50–400 ng) added to duplicate samples were 90–100%. Intra-assay coefficient of variation was 8.2% ($n = 16$), and interassay coefficient of variation was 9.7% ($n = 12$). Catecholamines (1 mg of either noradrenaline or adrenaline) or spermidine (10 μg) added to samples or blanks did not interfere with the fluorescence reading of histamine. If any 1-methylhistamine was to be taken up in the cellulose column (Tsuruta et al., 1981), this metabolite would not interfere with the interpretation of the results. Fluorometric determination of catecholamines was done by using a trihydroxyindole method routinely run in our laboratory (Anton and Sayre, 1962). Catecholamines were determined at the end of the experiments in the hearts of chronically sympathectomized and control rats, and in intact adrenals and in the remaining adrenal cortices of adrenal demedullated rats.

2.6. Adrenal demedullation

A group of rats was anesthetized with sodium pentobarbital, 40 mg/kg i.p. By way of a retroperitoneal approach, bilateral adrenal demedullation was performed in situ by making a small cut on the adrenal cortex and gently squeezing out the medulla. After surgical repair of the abdominal wall, 100 000 U procaine penicillin was administered s.c. The rats were used in the experiments 4 days after the operation. In order to check for the effectiveness of demedullation, at the end of the experiment the remaining adrenal cortices were removed for determination of catecholamines. An additional group of rats was sham operated: the adrenals were visualized but the medulla was not removed. The rest of the operation was similar to the one for adrenal demedullated rats.

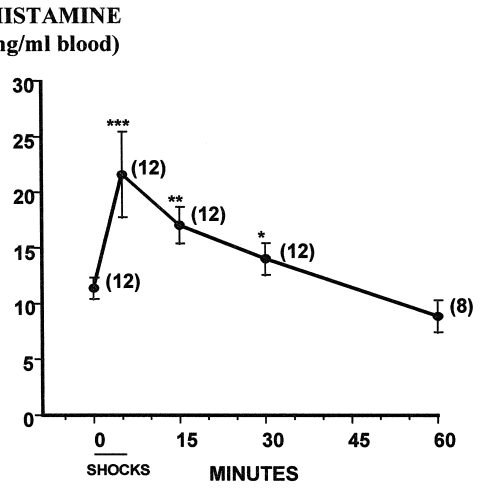

Fig. 1. Time-course of changes of rat blood histamine levels after brief inescapable footshocks. In parentheses, number of animals used for each point of the curve. (mean ± S.E.M.). Vs. control (0 time): $***P < 0.0005$; $**P < 0.01$; $*P < 0.05$.

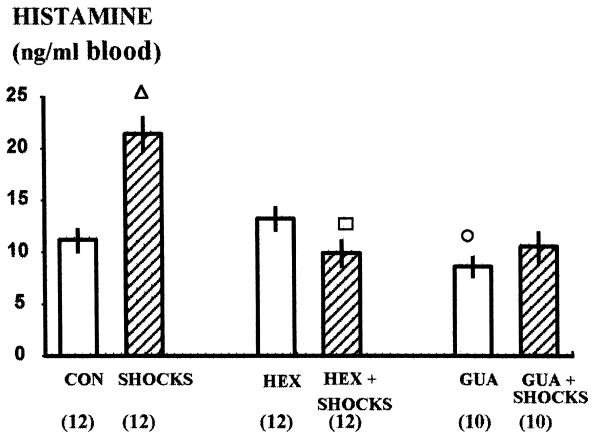

Fig. 2. Effect of either previous ganglionic blockade (hexamethonium, HEX, 10 mg/kg i.p.) or chronic sympathectomy (guanethidine, GUA, 25 mg/kg i.p. daily for 42 days) on the rise in blood histamine induced by brief inescapable footshocks. Vs control (CON): $^{\Delta}P < 0.0005$; $^{\circ}P < 0.025$. Vs. HEX: $^{\square}P < 0.05$.

2.7. Drug treatment

Whenever the ganglionic blocking agent hexamethonium bromide (Sigma) was used, the drug was administered at the dose of 10 mg/kg i.p. 10 min before shocks or 15 min before drawing blood samples in controls without shocks. In order to induce destruction of postganglionic sympathetic neurons with guanethidine sulphate (Ismelin, Ciba Pharmaceutical, Venezuela), young rats (less than one month old) were treated daily with the drug (25 mg/kg i.p.) for 42 days (Burnstock et al., 1971), and the rats were used at least 2 days after the last injection of guanethidine. The heart noradrenaline levels in these rats were undetectable in comparison to those in controls (703 $\pm$ 80, ng/g fresh tissue, mean $\pm$ S.E.M., $n = 6$).

2.8. Statistical analysis

Evaluation of differences was made by Student's *t*-test for independent means. $P < 0.05$ was considered significant.

3. Results

3.1. Time-course of changes of blood histamine levels induced by footshocks

Five-minute footshock stress induced a prolonged rise of blood histamine levels in the rat, with a peak at the end of the stimulation period which was almost twofold the baseline blood histamine level. Levels gradually approached normal 1 h after the beginning of stimulation (Fig. 1). The rise of blood histamine was not related to hemoconcentration (hematocrit %, mean $\pm$ S.E.M.: controls, 40.3 $\pm$ 0.99, $n = 9$: shocks, 42.4 $\pm$ 1.14, $n = 8$) or to a redistribution of leukocytes (leukocytes/mm^3, mean $\pm$ S.E.M., controls, 10 255 $\pm$ 777, $n = 9$; shocks, 10 000 $\pm$ 566, $n = 8$).

3.2. Effect of either ganglionic blockade with hexamethonium or guanethidine-induced sympathectomy on rises of blood histamine levels induced by footshocks

Ganglionic blockade with hexamethonium prevented the rise of blood histamine levels induced by footshocks. Furthermore, the blood histamine levels after hexamethonium plus shocks were lower than the ones after hexamethonium alone, but the former were not different from controls (Fig. 2).

Sympathectomy by itself also prevented the rise of blood histamine levels induced by footshocks (Fig. 2).

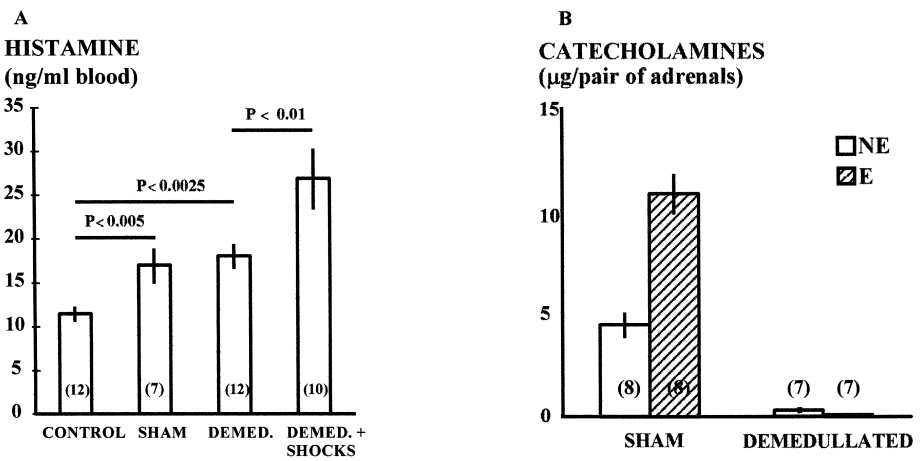

Fig. 3. (A) Effect of previous adrenal demedullation on the rise in rat blood histamine induced by brief inescapable footshocks. (B) Catecholamine concentrations in adrenals from sham-operated and demedullated (DEMED) rats. NE, noradrenaline. E, adrenaline.

Interestingly, baseline levels of blood histamine in sympathectomized rats were lower ($P < 0.025$) than the ones in controls with no treatment.

3.3. Effect of adrenal demedullation on rises of blood histamine levels induced by footshocks

Four days after adrenal demedullation, an increase in blood histamine levels in comparison to controls with no treatment was observed ($P < 0.0025$). This increase was similar to the one induced by the sham operation alone. Nevertheless, footshocks applied to demedullated rats induced a further increase in blood histamine levels above the increase elicited by adrenal demedullation alone (Fig. 3A; $P < 0.01$). This occurred in the presence of effective adrenal demedullation as judged from the finding of only traces of catecholamines in the remaining adrenal cortices of demedullated rats (Fig. 3B).

4. Discussion

Previous findings have shown that stressful situations cause an increase in blood histamine in both rats (Nakano and Suzuki, 1984; Head et al., 1985) and humans (Duner and Pernow, 1958; Hartley et al., 1981). All these situations are accompanied by an enhanced sympathetic activity. In in vitro experiments, stimulation of postganglionic sympathetic nerves to the perfused guinea pig (Gross et al., 1984) or rat (Harvey, 1979) heart causes an increase in histamine concentrations in the effluent. Also stimulation of postganglionic sympathetic nerves to the dog heart in vivo causes an increase in histamine levels in the coronary sinus blood (unpublished observations). In the present work, brief footshock stress in the rat, which causes a rise in blood catecholamines (Konarska et al., 1989), elicited both an immediate and pronounced increase in blood histamine levels, which was relatively long-lasting (Fig. 1). This increase seems to be dependent upon sympathetic activity, since previous ganglionic blockade or guanethidine-induced sympathectomy abolished this rise in blood histamine (Fig. 2).

In the vas deferens, histamine-containing neuronal pathways are independent of noradrenergic pathways (Campos, 1988). Thus, histamine levels in this organ are not modified when noradrenergic neurons are destroyed by guanethidine (Campos and Domínguez, 1995). This also occurs in the rat heart when the rat is treated with 6-hydroxydopamine, an agent that destroys noradrenergic nerve terminals but spares histamine levels in the heart (Harvey, 1978), emphasizing the independence of the two cardiac store sites as well. Therefore, the impairment of the rise in blood histamine level by guanethidine does not seem to be due to histamine depletion in rat tissues. In this sense, increases in histidine–decarboxylase activity induced by stimulation of the sympathetic pathway to the vas deferens are prevented by previous destruction of the noradrenergic neurons with guanethidine (Campos and Domínguez, 1995), which further shows that excitation of histamine-containing neurons depends upon the functionality of noradrenergic neurons.

Moreover, the increase in blood histamine levels during footshock stress appears to be dependent mostly upon the activation of postganglionic sympathetic neurons, because previous adrenal demedullation did not impair it (this article). In the same direction, the rise of rat plasma histamine induced by decapitation is not impaired by adrenalectomy (Head et al., 1985). It is worth noting that both sham-operated and adrenal-demedullated rats also showed an increase in blood histamine levels as compared to controls with no treatment (Fig. 3A), suggesting that other types of stress, such as the one induced by surgery, cause a relatively long-lasting increase in blood histamine levels in both rats (this article) and humans (Berger and Stopik, 1982). The fact that guanethidine-sympathectomized rats had lower baseline levels of blood histamine than the controls (Fig. 2) suggests that background sympathetic activity contributes, at least partly, to the maintenance of physiological blood histamine levels.

In agreement with this, the baseline levels of histamine in controls were lower than the ones reported by other authors (Friedman and Walker, 1969; Kowalewski et al., 1969; Nakano and Suzuki, 1984). All these authors have in common that they killed the rats by decapitation, which causes an intense nervous discharge and a sudden increase in blood histamine (Head et al., 1985), whereas we obtained our blood samples from the inferior vena cava after deeply anesthetizing the rats with sodium pentobarbital, and so under reduced nervous activity. In this respect, Head et al. (1985) have shown that plasma histamine levels are much lower in sodium pentobarbital-anesthetized rats when the blood is drawn from the inferior vena cava than when it was obtained after decapitation. This finding suggests that the excessive nerve activity induced by decapitation, presumably via the sympathetic system eliciting an increase in circulating noradrenaline (Head et al., 1985), causes a rise in blood histamine levels. In addition, increases in blood noradrenaline (Häggendal et al., 1970; Pernow et al., 1986) and histamine levels (Duner and Pernow, 1958; Hartley et al., 1981) under similar stressful situations (bicycle ergometer) have been observed in humans. It would be of interest to see whether or not there is a causal relationship between the increases in blood levels of both amines.

It appears to us that, as a compensatory mechanism, a peripheral reflex-releasing neuronal histamine could be evoked when sympathetic activity is enhanced (Campos and Briceño, 1992). In this regard, several pieces of evidence have shown the presence of histamine H_3 inhibitory receptors on peripheral sympathetic nerve terminals (Ishikawa and Sperelakis, 1987; Luo et al., 1991; Malinowska and Schlicker, 1991; Molderings et al., 1991).

These receptors could be involved in the inhibitory modulation of peripheral sympathetic activity. In this sense, brief (4 h) or prolonged (days) inhibition of L-histidine decarboxylase with alpha-fluoromethylhistidine, which presumably reduces peripheral neuronal histamine in its rapid turnover compartment as it occurs in the same compartment in the brain (Maeyama et al., 1983), causes an overall facilitation of sympathetic activity resulting, in the case of prolonged inhibition, in arterial hypertension and tachycardia (Domínguez et al., 1991; Campos et al., 1994, 1996). This facilitation, which appears to be peripheral in location, is similar to the local sympathetic facilitation induced by degeneration of histamine-containing neuronal pathways (local neuronal histamine depletion) in the vas deferens (Campos and Briceño, 1992).

Our findings suggest that peripheral neuronal histamine is reflexly released into the circulation during enhanced sympathetic activity and is mostly dependent upon the activation of postganglionic sympathetic neurons.

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