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The metabolism of histamine in rat hypothalamus and cortex after reserpine treatment

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ABSTRACT

The effect of reserpine on histamine (HA) and tele-methylhistamine (N^{τ}-MHA) in hypothalamus and cortex of rats was analyzed and compared to catecholamines. IP injection of reserpine (5 mg/kg) confirmed the effectiveness of reserpine treatment on noradrenaline and dopamine levels. Our in-vitro experiment with synaptosomal/crude mitochondrial fraction from hypothalamus and cortex confirmed that while mono amine oxidase (MAO) is an efficient metabolic enzyme for catecholamines, HA is not significantly affected by its enzymatic action. HMT activity after reserpine, pargyline and L-histidine treatment showed no differences compared to the control values. However HDC was significantly increased in both hypothalamus and cortex. In this study, *Ws/Ws* rats with deficiency of mast cells were used to clarify aspects of HA metabolism in HAergic neurons by eliminating the contribution of mast cells.

The irreversible MAO-B inhibitor Pargyline (65 mg/kg) failed to accumulate N^{τ}-MHA in the hypothalamus. However, when animals treated with reserpine and pargyline/reserpine were compared, the last group showed higher N^{τ}-MHA values ($p < 0.01$). Moreover, the precursor of HA, L-histidine (1 g/kg), produced an increase of HA in the hypothalamus to 166% and the cortex to 348%.

In conclusion, our results suggest that the effect of reserpine on the HA pools in the brain might be different. The neuronal HA pools are more resistant to reserpine as compared to those of catecholamine. Moreover, the HAergic pool appears to be more resistant to depletion than mast cells' pool, and thus HDC/HMT activity and its localization may play a key role in the understanding of HA metabolism in brain after reserpine treatment.

1. Introduction

Histamine (HA), a heterocyclic primary amine, is synthesized from the decarboxylation of the amino acid histidine by the enzyme L-histidine decarboxylase (HDC, E.C.4.1.1.22) (Watanabe et al., 1979). HA participates in local immune response (Hébert et al., 1980), circadian rhythm (Chu et al., 2004; Tuomisto, 1991), feeding behavior (Sakata, 1991; Yoshimatsu, 2008), motion sickness and locomotor activity (Clapham and Kilpatrick, 1994; Matsunaga and Takeda, 1991), memory formation (Blandina et al., 1996), cardiovascular control (Imamura et al., 1996), food and water intake (Lecklin et al., 1998) and gastric secretion (Furtani et al., 2003; MacIntosh, 1938), among others. HA has also been reported to act as a neurotransmitter in mammalian brain (Prell and Green, 1986; Schwartz, 1975; Wada et al., 1991). Two different pools containing HA in the brain have been identified – histaminergic (HAergic) neurons and mast cells (Garbarg et al., 1976; Panula, 1986; Watanabe et al., 1983).

The somata of HAergic neurons originate within the hypothalamic tuberomammillary nuclei, sending out their axons and innervating practically the entire brain and parts of the spinal cord (Oishi et al., 1983; Watanabe et al., 1984).

Two enzymes are known to participate in the degradation of HA: diamine oxidase (DAO; E.C. 1.4.3.6), within the digestive tract, to imidazole acetaldehyde, responsible for scavenging extracellular histamine after mediator release (Agúndez et al., 2012; Garcia-Martin et al., 2009); and histamine N-metyltransferase (HMT, E.C. 2.1.1.8) to N^{τ}-methylhistamine (N^{τ}-*MHA*), which is responsible for inactivating histamine in brain. Therefore, the fluctuation of HA and N^{τ}-MHA levels may provide an accurate information of HA turnover and HAergic neurons activity (Agúndez et al., 2012; Garcia-Martin et al., 2009; Oishi et al., 1983; Sugimoto et al., 1995).

HA has been thought to accumulate in secretory vesicles in neurons and granules of enterochromaffin-like cells in stomach and mast cells through the action of vesicle monoamine transporter VMAT, a member of the vesicular neurotransmitter transporter family, responsible for the translocation of monoamines (serotonin, dopamine, norepinephrine, and HA). Two isoforms, VMAT1 and VMAT2, have been identified in mammals; however, VMAT2 is the only isoform expressed in neuronal cells (Erickson et al., 1992; Liu et al., 1992).

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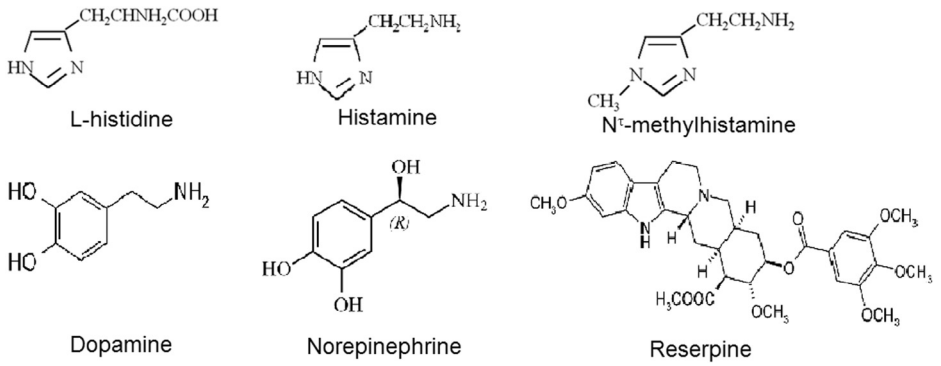

Fig. 1. Chemical structure of the neurotransmitters and reserpine. From left to right: The precursor of HA; HA; the metabolite of HA; catecholamines; reserpine.

In mammalian brain, monoamines are transported from the cytoplasm of the pre-synaptic nerves, via a proton electrochemical gradient generated by the vacuolar type H^+ -adenosine triphosphatase, into vesicles for storage and posterior release into the synaptic cleft via VMAT2 (Yelin and Schuldiner, 2002).

Reserpine, an indole alkaloid with antihypertensive and antipsychotic properties (Frize, 1954), interferes with the transport of monoamines by irreversibly inhibiting VMAT2 (Henry and Scherman, 1989). Therefore, its blocking action leads to an accumulation of amines in the cytosol and subsequent degradation by enzymes like MAO (monoamine O_2 oxidoreductase, EC 1.4.3.4). Brain and peripheral sympathetic nerves undergo a marked depletion of monoamines, resulting in symptoms of depression (Monoamine hypothesis) (Schildkuart, 1965) among others. Because of its numerous adverse-effects, nowadays reserpine is rarely used in human treatments. However, it has been proven to be a useful tool to study the mechanism of neurotransmitter transport into synaptic vesicles and represents a valuable instrument to understand the pathways of histamine metabolism in animal models.

In the present study, the effect of reserpine on HA and N^{τ}-MHA levels in hypothalamus and cortex of rats were investigated and compared to those of catecholamines (dopamine and norepinephrine). The chemical structure of reserpine and the neurotransmitters studied in this work are shown in Fig. 1. The effects of pargyline, clorgyline, deprenyl (L-deprenyl) and L-histidine, which all affect the synthesis and/or catabolism of HA and N^{τ}-MHA, were analyzed to reinforce our findings.

2. Materials and methods

2.1. Chromatographic instrumentation

HPLC experiments were performed according to Maldonado and Maeyama (2013).

The HPLC system consists of a model LC-100 AS micro LC pump (BAS, Tokyo, Japan), a vacuum degasser Shodex Degas (Showa Denko, Japan), a Model 7125 injector (Reodyne, Cotati, CA, USA), a reversed phase analytical column (TSK-gel ODS 80Ts,5 μm, 4.6 mm i.dx 150 mm; Tosoh, Tokyo, Japan), and a potentiostat ALS/CHI, Electrochemical Analyzer Model 800 (BAS, Tokyo, Japan) with a glassy carbon electrode. The column was eluted with a 30% methanol in 50 mM potassium phosphate buffer (pH 6.50) containing 0.5 mM Na_2-EDTA at a flow rate of 500 μl/min and the detector output current was monitored at a potential of +0.75 V. Peak areas (expressed as charges) and sample concentrations were collected with an ALS800a EC MFC (CH Instruments, Inc. USA).

2.2. Reagents and materials

HA phosphate, OPA, sodium borate ($Na_2B_4O_7$), Na_2SO_3-anhydrous, dopamine, norepinephrine and L-histidine hydrochloride monohydrate were purchased from Wako Pure Chemicals (Osaka, Japan). N^{τ}-MHA, pargyline hydrochloride, deprenyl (Selegiline), clorgyline, propionic acid and reserpine were purchased from Sigma-Aldrich (St Louis, MO, USA). Viva pure sulphonic acid (S) R-CH_2-SO_3-Na^+ strong acidic cation exchange (high binding capacity) spin columns were purchased from Sartorious Stedim Biotech.

OPA- Na_2SO_3 derivatization solution was prepared combining 25 μl of 0.2 M OPA in methanol and 25 μl of 1 M Na_2SO_3 with 950 μl of 0.1 M sodium borate buffer (pH 9.65).

2.3. Animals

Male Wistar and *Ws/Ws* rats (220–250 g) were used in this study.

The mast cell deficient *Ws/Ws* rat model is characterized by a homozygosis for a 12 base deletion in the tyrosine kinase domain of the c-kit receptor gene, commonly known as the white-spotting locus. The c-kit receptor induces mast cell proliferation and differentiation when activated by stem cell factors (Tsujimura et al., 1991).

The animals were housed at a constant temperature of 22 ± 2 °C with an automatically controlled 12:12-h light-dark cycle (light on at 7:00 am). Food and water were available ad libitum. The experimental protocols were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was reviewed and approved by the Committee on the Ethics of Animal Studies of Ehime University. Surgeries were performed under IP injection of sodium pentobarbital (Nembutal) 50 mg/kg, and all efforts were made to minimize suffering.

2.4. Animal drug pre-treatment

Drugs were administrated by IP injection. Reserpine, 5 mg/kg, was injected 24 hours before euthanasia; pargyline, 65 mg/kg, was injected 90 min before euthanasia; and L-histidine, 1 g/kg, was injected 3 hours before euthanasia. Euthanasia was carried out by decapitation under previous deep sedation with Nembutal.

2.5. Pre-purification of HA, N$^{\tau}$-MHA and catecholamines from biological samples

HA and N^{τ}-MHA were purified using a Vivapure column. This Vivapure mini H filter with 3–5 μm pore size was washed with 0.5 ml of 2 M HCl and 0.5 ml of 1 M NaOH, twice and then equilibrated with