Notes

[Chem. Pharm. Bull. 22(4) 950—952 (1974)]

UDC 547.781.5.08 : 615.31.033.076.9 : 543.426

Regional Distribution of Histamine measured by a Fluorometric Determination in the Dog Brain

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(Received June 20, 1973)

The fluorometric determination of brain histamine by *o*-phthalaldehyde (OPT) method has been revealed to be interfered by the presence in extracts of brain of some substances, such as slow reacting substance and spermidine, which yield fluorescent compounds after reaction with OPT.[2] Therefore, various ion exchange chromatographies were used to eliminate interfering substances in the brain extract. Kremzner and Pfeiffer[3] have shown that brain histamine is separated from the major interfering substance, spermidine, by a phosphorylated cellulose column and the specificity was confirmed by Medina and Shore.[4] Green and Erickson[5] have reported a histamine content of the rat brain after separation of histamine by use of a Dowex 50W-X4 column, while we observed that unknown substance which interfered the histamine determination might be included in the solution eluted from the Dowex column.

Present paper describes an improved method to separate a small quantity of brain histamine for the fluorometric determination from the interfering substance by a Dowex 50W-X4 column and the results obtained using the method about a regional distribution of histamine in the dog brain which can be considered to be more precise than that hitherto reported.

Material and Method

Male albino rats of Donryu strain weighing 200 to 300 g and mongrel dogs weighing 10 and 13 kg were used. They were sacrificed by decapitation (rat) or by perfusion with ice-cold Krebs–Ringer solution containing 170 μM EDTA under anesthesia with thiopental sodium 35 mg/kg *i.v.* (dog). The brain was immediately isolated, weighed and either processed or frozen in liquid nitrogen and stored at −20°. The bilateral olfactory bulbs and the cerebellum were removed from the rat brain. The dog brain was divided into several regions in ice-cold Krebs–Ringer solution. The fluorometric measurement was performed by Hitachi MPF-2A type of spectrofluorophotometer.

1) **Analytical Reagents**——Histamine dihydrochloride, diphenhydramine hydrochloride and atropine sulfate were used in analytical grade. OPT recrystalized from *n*-heptane was solved in methanol (0.5 w/v%). *n*-Heptane and isoamyl alcohol were washed with 1N HCl, 1N NaOH and distilled water. A Dowex 50W-X4 column (200 to 400 mesh, wet weight 750 mg, dimensions 37 $mm^2 \times 20$ mm) was used after washing according to the method described by Bertler, *et al.*[6]

2) **Analytical Procedure**——Tissues were homogenized in 3 to 5 volumes of ice-cold 0.4N perchloric acid and the homogenate was allowed to stand at 4° for 30 min before centrifugation at 9000 rev./min in the refrigerated centrifuge for 10 min. The supernatant was decanted and the residue of the tissue was re-extracted with 2 ml of 0.4N perchloric acid. The extracts were combined and added EDTA solution

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2) E.A. Carlini and J.P. Green, *Br. J. Pharmac.*, **20**, 264, 1963.
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(final concentration, EDTA 1 mg/ml). Seven ml of extract was carefully adjusted to pH 12.0 with 10N NaOH using pH meter and the resulting solution was transfered into 50 ml glass-stoppered centrifuge tube containing 4.5 g of NaCl and added 23 ml of isoamyl alcohol. This mixture was shaken for 15 min, and centrifuged at 3000 rev./min for 3 min in a centrifuge to separate the organic phase. Isoamyl alcohol in a volume of 20 ml was transfered into a 50 ml glass-stoppered centrifuge tube containing 3 ml of 0.5N HCl and 1.5 ml of *n*-heptane. The tube was shaken for 15 min and centrifuged at 3000 rev./min for 2 min. 2.5 ml of 0.5N HCl layer was taken and diluted with 20 ml of 0.1M phosphate buffer (final pH 6.0). The resulting solution was passed through the Dowex column. After rinsing with 20 ml glass-distilled water, 15 ml of 0.8N HCl and 5 ml of 1.6N HCl, elution was performed with 5 ml of 1.6N HCl containing 0.05% ascorbic acid.

The elute in a volume of 2 ml was taken for analysis according to the procedure of Shore, *et al.*,[7] as modified by following workers[8] and ourselves. A 1.0 ml portion of the elute was made alkaline by addition of 0.4 ml of 10N NaOH and added 0.1 ml of OPT reagent in a ice-cold bath with constant stirring. The test tube containing the resulting solution was transfered into a water bath at 25° and shaken exactly for 4 min. Then the tube was transfered into the ice-cold bath and the solution was acidified with 1.0 ml of 3M H_3PO_4. The fluorescence spectrum from 400 through 500 mμ (peak at 450 mμ) at 350 mμ of activation wave length was recorded. Tissue blanks were measured by reversing the order of addition of the OPT reagent and H_3PO_4. The fluorescence intensity was proportional to histamine concentration in the range from 3 to 500 ng/ml (maximum concentration examined).

3) **Recovery and Specificity of the Procedure**——The recovery of 100 μg of authentic histamine added to each of 4 rat brain extracts, each equivalent to 1.4 g of wet brain tissue of the rat, was 72.9±1.6% (mean ±S.E.M.). The endogenous histamine content measured in 4 replicates of rat brain extract was reproducible within 2%. In two experiments of the dog brain, the residual elute after the fluorometric measurement was evaporated *in vacuo*, suspended in Krebs–Ringer solution, and assayed with the guinea-pig ileum that was bathed at 32° in a 8 ml chamber with Krebs–Ringer solution (bubbled by 95% O_2 and 5% CO_2) containing atropine (10^{-7} g/ml). The guinea-pig ileum was contracted by extract of the dog brain immediately after the addition to the bath. The contration by was blocked by 10^{-8} g/ml of diphenhydramine hydrochloride. 5-Hydroxytryptamine (5-HT) antagonist was not used in this experiment, since authentic 5-HT added into the brain extract could not be detected in the acid elute when the fluorometric measurement was tried by the method of Andén and Magnusson.[9] The values of histamine concentration in the dog brain calculated from bioassay were the same order as those measured by the fluorometric method.

4) **Comment**——When 10, 20, 40, and 80 ng of authentic histamine were added into 4 replicates of the rat brain homogenate and an analysis was performed by the method of Green and Erickson,[5] a linear relation between added histamine concentration and fluorophor strength was not observed, which indicated that the interfering substance was included in the Dowex elute. This problem was improved by the present procedure described above. The elution pattern with 1.6N HCl of histamine adsorbed on Dowex column was varied in the Lot No. of the resin. The addition of ascorbic acid into the Dowex elute resulted in good reaction of fluorophor formation by histamine and OPT, while the mechanism of the reaction was not known.

Result and Discussion

n-Butanol or isoamyl alcohol extract of brain contains spermidine along with histamine. Spermidine reacts with OPT to produce a fluorophor with histamine-like properties.[2,10] Various ion-exchange chromatographies have been used to separate histamine from interfering substance(s) in brain extract. We found that the purification of brain extract with a Dowex 50W-X4 column was insufficient for this purpose. Therefore, we tried to purify brain histamine using isoamyl alcohol extraction in addition to the Dowex column separation, and made much improvement for the fluorometric determination of brain histamine.

The histamine content in the rat brain without the cerebellum was 78±5 ng/g (mean± S.E.M., $n=6$), which was similar to those reported by previous workers.[8] Brain histamine was separated from interfering substance(s) by the combination of the extraction with isoamyl alcohol and the purification with the Dowex column. The specificity, sensitivity and reproducibility of the present method were no less better than those described by other workers,

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so far as the fluorometric measurement was used. However, the maximum sensitivity was about a tenth in comparison with the enzyme-isotopic method.[11]

In the dog brain, the highest concentration of histamine was found in the posterior hypothalamus (819 and 641 ng/g, $n=2$). Following values were obtained in other regions: 498 and 424 ng/g in the anterior hypothalamus, 331 and 248 ng/g in the thalamus, 151 and 143 ng/g in the caudate nucleus, 153 and 138 ng/g in the midbrain and 129 and 101 ng/g in the area postrema. The low concentrations (<100 ng/g) were found in the occipital cortex, the cerebellum, the pons, the medulla and the spinal cord. The similar distribution of histamine was reported in the human brain as well as the experimental animal brain.[12] Schwartz, *et al.*[13] found the highest histidine decarboxylase activity in the hypothalamus of the rat brain. Adam[14] has reported the highest concentration of histamine in the area postrema of the dog brain, but our result showed a low value. The reason for this discrepancy is not clear. The present finding and the results reported by previous workers revealed that 1) the hypothalamus contained the highest concentration of histamine in the brain of the experimental animal, 2) histamine in the rat brain was largely found in particles with sedimentation properties in sucrose gradients similar to synaptic vesicles storing noradrenaline and 5-hydroxytryptamine,[15,16] 3) the histamine content in the cat brain was altered after the treatment with drugs,[17] and 4) the formation of histamine from histidine was demonstrated in the cat brain.[18] These facts may indicate non-mast cell site for histamine storage and some role of histamine in the regulation of neuronal activity in the hypothalamus, where mast cells are scarce if present at all.

Acknowledgement The authors are indebted to Miss Keiko Sato for preparing the manuscript.

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[Chem. Pharm. Bull. 22(4) 952—957 (1974)]

UDC 546.32.09 : 615.31'1.015.42

Factors influencing Absorption and Excretion of Drugs. II.[1] Effect of Potassium Ion on *in Situ* Rat Intestinal Absorption of Several Drugs

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(Received July 18, 1973)

Some investigators have reported that various monovalent cations such as K^+, NH_4^+, Li^+, and guanidine$^+$ present in a drug-buffer solution can have a marked influence on the passive transfer of several drugs across the everted rat intestine. For example, Nogami, *et al.*[3]

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