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Variation in gastric histamine levels and effects of histidine decarboxylase inhibition in rats from different sources

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IN AN earlier communication[1] it was reported that both 4-bromo-3-hydroxybenzyloxyamine (NSD-1055) and a hydrazino analog of histidine (MK-785) were potent inhibitors of specific histidine decarboxylase *in vitro*. Parenteral administration to rats of either of these substances was followed by rapid depletion of a rapidly turning-over pool of histamine in gastric mucosa.

Subsequently, in a different laboratory, the author has continued to investigate the effects of these drugs on histamine metabolism. Preliminary experiments failed to confirm the results of the previous report; neither NSD-1055 nor MK-785 produced any change in histamine levels in stomach or any other tissue studied. Experiments designed to assess possible influences of conditions of animal housing, diet, environmental temperature, seasonal variation, methods of preparing and administering the drugs, and methods of processing tissue for histamine assay failed to yield an explanation for the unexpected results. The last factor to be considered was the source of the experimental animals. In rats obtained from each of two different breeders it was possible to demonstrate that, as reported previously, administration of NSD-1055 was followed by rapid depletion of histamine levels in gastric tissue.

It is generally known that animals of different species differ importantly in several aspects of metabolism and in their responses to various drugs. Furthermore, different strains within the same species may have metabolically or physiologically unique properties. The purpose of this communication is to emphasize that closely related animals within a species may differ greatly and that this may introduce an important, and sometimes overlooked, source of experimental variation.

The animals used in the studies reported in this paper were female rats weighing 150 to 200 g; all were derived originally from Sprague-Dawley stock. They were obtained from the following sources: "Sprague-Dawley" rats from Blue Spruce Farms, Inc., Altamont, N.Y. (BSF); "CFE" rats from

Carworth, Inc., New City, N.Y.; and "CD" rats from Charles River Laboratories, Inc., North Wilmington, Mass. Animals were permitted to ingest nothing but water for 14 to 16 hr before study. NSD-1055 (4-bromo-3-hydroxybenzyloxamine dihydrogen phosphate) or MK-785 [D-2-hydrazino-3-4(5) = imidazole propionic acid] was dissolved in aqueous solution immediately before use; the intraperitoneal dose was 200 mg drug base/kg. Animals were sacrificed by decapitation without anesthesia; the glandular portion of the stomach was removed, washed with cold water, and placed on cracked ice for less than 30 min or frozen for less than 48 hr prior to assay. Assay of histamine was done by the fluorometric method of Shore *et al.*[2] as modified by Levine *et al.*[1]

The critical results of these studies are listed in Table 1. In the BSF rats, no alteration in gastric histamine levels was detected at 3, 6, or 24 hr, respectively, after administration of NSD-1055 or at either 3 or 24 hr after administration of MK-785. The doses (200 mg/kg) of each drug were twice those required to produce maximal effects in NIH rats.[1] Since the supply of MK-785 is limited, the remainder of the studies were done only with NSD-1055.

TABLE 1. GASTRIC LEVELS OF HISTAMINE BEFORE AND 3 HR AFTER INTRAPERITONEAL ADMINISTRATION OF NSD-1055

	Type of rat			
	NIH*	BSF	CFE	CD
Control	19·7 ± 2·7†	37·6 ± 4·3	41·1 ± 4·2	19·5 ± 3·0
NSD-1055 (200 mg/kg)	13·3 ± 1·6	39·2 ± 5·6	29·3 ± 3·7	12·8 ± 1·9
P	<0·001‡	>0·05	<0·001	<0·001

* Initials designate rats obtained from different suppliers; all were derived originally from Sprague-Dawley stock. Suppliers were: NIH, National Institutes of Health; BSF, Blue Spruce Farms; CFE, Carworth, Inc.; and CD, Charles River Laboratories. Data on NIH rats were taken from study reported previously[1] and included here for purposes of comparison. Observations in NIH rats were not repeated as part of the present study.

† Each number represents the mean histamine level (μg/g ± 1 standard deviation) of observations made in at least ten rats.

‡ Probability of chance occurrence of difference from control.

In the CD rats and CFE rats, as in the NIH rats, administration of NSD-1055 was followed by depletion of histamine in gastric tissue. Also, as in the NIH rats, this effect developed rapidly and was well established within 3 hr. A further point of interest was the great difference in histamine levels in control rats from different sources. Whether or not the drug caused histamine depletion, and the magnitude of that depletion, did not appear to be determined by the control value.

There are two significant points to be made from these studies. First, in rats obtained from four different sources but all derived originally from Sprague-Dawley stock, mean control gastric histamine levels varied from 19·5 to 41·1 μg/g, while the standard deviation within any group did not exceed 4·3. Second, in three of the four groups, administration of NSD-1055 was followed promptly by significant depletion of histamine from gastric mucosa, whereas in another group there was no effect whatever.

The reason for these variations in gastric histamine levels and in the effects of administration of histidine decarboxylase inhibitors cannot be defined with the aid of data that are now at hand. A systematic study of histamine biosynthesis and catabolism in these rats probably would yield valuable information on genetic control of, and environmental influences on, the metabolism of histamine.

This sort of variation among experimental animals that we presume either to be identical or very closely related may account for some failures to reproduce the findings of another laboratory, even though the published experimental design is followed carefully. Apparently, it is not sufficient to

identify experimental animals as Sprague-Dawley rats or Hartley guinea pigs or some other similar designation. It is also necessary to identify the supplier of the animals.

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Cardiac myofibrillar ATPase activity in hypophysectomized or thyroidectomized rats*

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RECENTLY it was shown that there is a significant reduction in adenosinetriphosphatase (EC 3.6.1.3, ATPase) activity of cardiac myofibrils prepared from hypophysectomized or thyroidectomized rats.[1] The hydrolysis of ATP by the contractile protein of muscle might be the force-generating reaction that is ultimately linked to the velocity of shortening or the rate of isometric tension development. Thus, there is at least a plausible explanation for the marked reduction in cardiac output in hypophysectomized or thyroidectomized animals.[2, 3] The experiments described herein were designed to test whether thyroxine and growth hormone are effective in restoring the cardiac myofibrillar ATPase activity to normal. The results have important implications with regard to recent experiments on the physiological effects of these hormones.

MATERIALS AND METHODS

Female rats, Sprague-Dawley strain, were purchased from Hormone Assay Laboratories, Chicago, Ill. They were given Purina laboratory chow and water *ad libitum*. Two series of experiments were done.

Series I. Thyroidectomized rats, two days post-operative, weighed 102–134 g on arrival. They were kept for 2 months and then divided at random into two groups, one of which was started on thyroxine treatment. The dose of thyroxine (Na, L-thyroxine dissolved in 0·9% NaCl–0·001 N NaOH) was 8 μg/rat per day. Subcutaneous injections were given 6 days a week. ATPase assays were done between days 22 and 35 of treatment. Normal rats were received about 2 weeks before the ATPase assays were begun. They weighed 136–150 g, and at the time of sacrifice their final weight was approximately the same as the untreated, thyroidectomized rats. Therefore, the control rats in this series were slightly younger than the experimental rats.

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