rations; (ii) by reference to electrophysiological findings on bivalve visceral muscle; and (iii) by consideration of comparative anatomy.

The general pattern of the pharmacology of the *Katelysia* rectum is similar to that of *Mercenaria mercenaria* (*1*), *Tapes watlingi* (*2*), and other clams [*Anodonta grandis, Mercenaria campechiensis, Macrocallista nimbosa, Mytilus edulis,* and *Mytilus planulatus* (*5*)]. The depressor and excitor actions of ACh, and especially the blockade of 5HT by anticholinergic agents, were the crucial pharmacological findings upon which Greenberg and Jegla (*1*) and Phillis (*2*) proposed their model control systems for the clam rectum. The models differed in detail, but both included the possibility of excitation and inhibition by ACh at specific cholinergic sites in neuromuscular junctions. Both allow for excitation by 5HT either at sites on the muscle (*1*) or by stimulation of excitor cholinergic fibers (*1, 2*). Depression by 5HT occurred in *Mercenaria* and was explained by stimulation of depressor neurons (*1*). A 5HT depressor component is also built into the *Tapes* model. A feature of both models is that the observed response to any dose of 5HT or ACh is the sum of at least two antagonistic components. Consequently, the magnitude and direction of the observed response depends upon the dose and relative effectiveness of the drug at the antagonistic receptors. The relative smallness of the tonic excitation by ACh and the large persistent depressor effect on the rectum of *K. scalarina* could be due to a greater sensitivity to ACh at the depressor neuromuscular junction than at the excitor. Furthermore, this relatively large depressor effectiveness must be greater than that for *K. rhytiphora.* It is only by even more complex and unjustifiable theoretical contortions that the phasic response of the *K. scalarina* rectum to 5HT, but not to ACh, can be explained by these models.

Neither model can explain the phasic response to ACh. Greenberg and Jegla (*1*), on pharmacological evidence, suggested different mechanisms for the tonic and phasic responses of *M. mercenaria.* Prosser *et al.* (*3*) were able to produce these effects, not only by application of ACh, but also by direct electrical stimulation of the rectum and by indirect stimulation through the visceral ganglion. They were also able to separate the tonic and phasic responses: a spike preceded the phasic response but not the tonic; the tonic and phasic excitation properties were different; and the phasic, but not the tonic, contraction was abolished in high concentrations of potassium. They suggest, for the rectum of *Spisula solidissima,* not only a tonic excitor and depressor, but also a separate phasic excitor innervation. It is not likely that *K. rhytiphora* would have this phasic innervation while *K. scalarina* does not.

The phasic contraction, and its associated spike, is probably a function of the synchrony of contraction of muscle cells in the rectum. One would expect that such structural features as density and distribution of connective tissue, extent and type of contact between muscle cells, and density and type of innervation might be dissimilar in the rectums of the two species. Nielsen (*4*) found the histology of the entire alimentary tracts of the two species, as well as the gross anatomy of the nervous systems, to be similar.

However, a further study, restricted to the rectum and using ultramicroscopic (*7*) and established histochemical (*8*) techniques, might reveal a structural basis for the pharmacological differences.

The rectums of other pairs of intrageneric species of bivalves have been examined [*Mercenaria mercenaria* and *M. campechiensis*; *Mytilus edulis* and *M. planulatus* (*5*)]; in neither case were there discernible differences in pharmacology. However, the two sympatric species of *Katelysia* have minimal evolutionary variability, and also show very different physiological properties. Therefore, they are potential and unique tools for the investigation of these properties.

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9. Benzoquinonium chloride (Mytolon) was provided by A. M. Lands, Sterling-Winthrop Research Institute.
10. I thank Prof. G. Burnstock for providing space and equipment in his laboratory and C. F. McMurrich and S. Kirby for technical assistance. Work supported by NIH (HE09283) and by an NSF senior postdoctoral fellowship.
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4 August 1966

## Histamine Synthesis in Man: Inhibition by 4-Bromo-3-Hydroxybenzyloxyamine

Abstract. *Oral administration of 4-bromo-3-hydroxybenzyloxyamine to normal humans resulted in decreased urinary excretion of histamine; the normal increase in urinary levels of histamine after oral histidine loading was prevented. In two patients having systemic mastocytosis, additional evidence of inhibition of biosynthesis of histamine included marked reduction in symptoms attributed to histamine, and prevention of symptomatic exacerbation associated with histidine loading.*

Histamine is a naturally occurring amine that possesses a wide range of potent pharmacologic effects. Investigators have proposed important roles for this substance in the mediation of various physiologic functions (*1*) and in the pathogenesis of many human diseases (*2*). Thus a drug that effectively inhibits the biosynthesis of histamine in man should be of interest both as a tool for physiologic investigations and as a potential therapeutic agent.

Histamine is synthesized in mammalian tissues by decarboxylation of the precursor amino acid, histidine; its biosynthesis differs from that of other biogenic amines, such as norepinephrine and serotonin, in at least two important respects: firstly, decarboxylation, being the only enzymic process involved, is rate-limiting; secondly, at least in the rat, biosynthesis of histamine is catalyzed by a specific histidine decarboxylase (*3*). Thus inhibition of histidine-decarboxylase activity may be expected to result in more or less spe-

cific depletion of histamine from tissues.

It was reported (*4*) that 4-bromo-3-hydroxybenzyloxyamine (NSD-1055, CL-54998) was a potent inhibitor of both the specific histidine decarboxylase and the nonspecific aromatic L-amino acid decarboxylase. These findings were confirmed by Levine *et al.*, who showed also that administration of this drug to rats promptly resulted in depletion of the rapidly turning-over pool of histamine from tissues, and in decreased urinary excretion of histamine (*3*). Subsequently it was shown that such depletion of tissue stores of histamine resulted in inhibition of at least one physiologic function of histamine—gastric secretion of acid in the rat (*5*); inhibition was not associated with antagonism of the effects of administered histamine.

I studied the effects of NSD-1055 in humans on four subjects who were hospitalized continuously in metabolic research wards (*6*): two men lacking known abnormality in histamine metabolism and two women having extensive systemic mastocytosis, a disease characterized by abnormal proliferation of mast cells and excessive production of histamine (*2*). Both women had extensive infiltration of mast cells in the skin as well as in internal viscera; their symptoms attributed to histamine included hives, itching, local erythematous and generalized flushing reactions, headaches, abdominal cramps, nausea, vomiting, and diarrhea. Subjects were unrestricted as to hospital diet and physical activity.

Urine was collected, stored, and assayed fluorometrically for histamine content by a method described (*7*). Oral loading with histidine was accomplished by feeding the subjects L-histidine–HCl, suspended in applesauce, in an amount equivalent to 100 mg of free amino acid per kilogram of body weight. NSD-1055 was administered orally in gelatin capsules, each containing 200 mg of the drug as the dihydrogen phosphate salt. Dosage was divided as follows: 400 mg/day as 200 mg every 12 hours and 600 or 1200 mg/day as 200 or 400 mg every 8 hours, respectively. In the patients having systemic mastocytosis, but not in the control subjects, treatment with NSD-1055 was followed for a period of 1 week by administration of an equal number of placebo capsules that were identical in appearance with those containing the drug.

Table 1. Effects of oral administration of NSD-1055 on urinary excretion of histamine. "Base line" values are means, ± S.D., of values during the week on days on which histidine was not administered. "With histidine load" values were obtained on day 5 or 6 on weeks 2 to 6 and on various days during the control period preceding treatment; they are reported as single observations.

| Week of study | NSD-1055 dosage (mg/day) | Urinary histamine (μg/24 hr) | |
|---|---|---|---|
| | | Base line | With histidine load |
| | *M.P.; male control* | | |
| 1 | 0 | 27 ± 7.2 | 48, 60, 42 |
| 2 | 600 | 10 ± 4.1 | 25 |
| 3 | 1200 | 9 ± 5.0 | 10, 8 |
| 4 | 0 | 13 ± 3.5 | 49 |
| 5 | 0 | 35 ± 7.3 | 75 |
| | *C.M.; male control* | | |
| 1 | 0 | 32 ± 5.4 | 67, 157 |
| 2 | 600 | 11 ± 3.2 | 20 |
| 3 | 1200 | 16 ± 2.1 | 13 |
| 4 | 0 | 35 ± 13 | 81 |
| | *L.D.; female having mastocytosis* | | |
| 1 | 0 | 72 ± 11 | 94, 116 |
| 2 | 600 | 82 ± 15 | 73 |
| 3 | 600 | 76 ± 11 | 74 |
| 4 | 0 | 119 ± 25 | 90 |
| | *M.C.; female having mastocytosis* | | |
| 1 | 0 | 74 ± 16 | 65, 84 |
| 2 | 600 | 61 ± 25 | 70 |
| 3 | 400 | 59 ± 8.0 | 64 |
| 4 | 400 | 91 ± 25 | 43 |
| 5 | 400 | 22 ± 10 | 16 |
| 6 | 0 | 51 ± 23 | 52 |

The effects of NSD-1055 administration and histidine loading on urinary excretion of histamine are summarized in Table 1. In the control subjects, histidine loading resulted in markedly increased urinary excretion of histamine. Decreased urinary excretion of histamine was evident in both men on the 2nd day of NSD-1055 administration; the decrease in the effect of histidine loading on urinary histamine appeared during the 1st week of drug administration and became complete during the 2nd week. Neither man noted any subjective effects of the drug or of histidine loading.

The findings from the patients having mastocytosis were different. Histidine loading was associated with marked increase in the frequency and severity of all symptoms attributed to histamine, but with little or no increase in urinary histamine. During administration of NSD-1055 the following sequence of events was observed. By day 4 to 5 there was significant reduction of all symptoms attributed to histamine; on day 6 histidine loading was repeated, with no worsening of symptoms in either patient; spontaneous symptoms almost completely disappeared by day 8. Relief from spontaneous symptoms, as well as from those associated with histidine loading, (repeated on day 5 or 6 of each week), persisted throughout treatment with NSD-1055. In patient M.C., urinary excretion of histamine did not decrease substantially until the 4th week of drug administration; In L.D., during a shorter period of NSD-1055 trial, urinary histamine levels did not decrease. When drug administration was stopped, however, urinary excretion of histamine increased substantially.

Substitution of placebo for NSD-1055 in both patients having systemic mastocytosis was followed within 48 hours by the return of symptoms: L.D. reported that, on days 3 to 5 of placebo administration, symptoms were more severe than they had been before treatment with NSD-1055; during the placebo period histidine loading again produced severe symptoms in both patients.

Frequent appropriate laboratory tests revealed no evidence of toxic effect on renal, hepatic, or cardiac function, or on counts of red or white blood cells. The only symptomatic evidence of adverse effect was reported by M.C.: on each of two trials more than 1 month apart, administration of NSD-1055 in a total dose of 600 mg/day was associated with nausea and vomiting. On the second of these occasions these symptoms disappeared when the dosage was reduced to 400 mg/day.

This is apparently the first demonstration of inhibition of biosynthesis of histamine in man. The finding that treatment with NSD-1055 of patients having systemic mastocytosis resulted in marked symptomatic relief within a relatively short period seems to have dual significance. Firstly, it supports the belief that these symptoms are effects of histamine; secondly, it encourages investigators to undertake therapeutic trials of NSD-1055 and other drugs that inhibit histidine-decarboxylase activity in other more common diseases in which excessive effects of histamine have been implicated—in asthma and other allergic disorders, peptic ulcers, and vascular headaches (*2*).

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8 August 1966

## Phytohemagglutinin: Inhibition of the Agglutinating Activity by N-Acetyl-D-Galactosamine


Abstract. *The effect of simple sugars on the agglutinating activity of phytohemagglutinin was studied.* N-*Acetyl*-D-*galactosamine selectively inhibits the agglutination of leukocytes and erythrocytes by phytohemagglutinin.*


Phytohemagglutinin (PHA), an extract of the red kidney bean *Phaseolus vulgaris,* agglutinates leukocytes and erythrocytes (*1*); it also stimulates lymphocytes in vitro to differentiate into large cells capable of undergoing mitosis (*2*).

It has been reported that agglutination caused by fava-bean extract, another plant hemagglutinin, could be inhibited by D-glucose, D-fructose, and maltose (*3*). Agglutination of lymphocytes by PHA is inhibited by coating the cells with Vi antigen from *Salmonella* (*4*), a substance containing *N*-acetyl-D-galactosaminuronic acid (*5*). These studies have raised the possibility that sugars may play a role in the interaction between PHA and mammalian cells. We have investigated the effect of certain sugars which occur as constituents of mammalian cell surfaces on the agglutination of cells by PHA.

The contents of each vial of PHA (*6*) were dissolved in 5 ml MEM (*7*), the diluent routinely used in our experiments, and this was used as a stock solution from which serial dilutions were made. Sugars were obtained commercially and made up in stock solutions of 100 mg per milliliter of MEM. Erythrocytes were obtained from man, rabbits, and rats by drawing blood into a syringe containing heparin. Cells from rat thymus, rabbit thymus, and human tonsil were obtained by gently teasing these tissues in MEM and filtering the suspension through a thin layer of glass wool. Rat thoracic duct cells were obtained by conventional techniques (*8*). For agglutination, cells were washed three times in MEM, and 0.2 ml of a suspension ($10^7$ cells/ml) was incubated with 0.1 ml of PHA and 0.25 ml of sugar solution. After 3, 10, and 30 minutes at 37°C, samples of the reaction mixtures were streaked on a slide and examined microscopically (100×). The degree of agglutination was graded on a 0 to 4 scale. Test samples from which PHA or sugar, or both, had been omitted were included as controls in each experiment.

*N*-Acetyl-D-galactosamine (5 mg/ml) selectively inhibits the agglutination of rat thoracic-duct lymphocytes by PHA (Table 1). A lesser degree of inhibition was observed with 10 mg of the *N*-acetyl-D-galactosamine per milliliter. The other sugars, in concentrations of 10 to 100 mg/ml, showed no consistent inhibition at 30 minutes. L-Fucose, however, at a final concentration of 50 mg/ml, showed slight and variable inhibition after 10 minutes of incubation. Cells freshly agglutinated by PHA could be disassociated by the addition of *N*-acetyl-D-galactosamine (50 mg/ml). *N*-Acetyl-D-galactosamine selectively inhibited agglutination by PHA of rat thymus, rabbit thymus, and human tonsil cells, and also of human (Table 2), rat, and rabbit erythrocytes.

Table 1. Inhibition of agglutination of rat thoracic duct cells by PHA with *N*-acetyl-D-galactosamine. In each reaction mixture the final concentration of the sugar was 50 mg/ml. The readings were taken 10 minutes after incubation at 37°C was begun.

| Sugar added | Agglutination at dilution of PHA | | | | | |
|---|---|---|---|---|---|---|
| | 1:25 | 1:50 | 1:100 | 1:250 | 1:500 | 1:750 |
| None | +++ | +++ | ++ | ++ | ++ | + |
| *N*-Acetyl-D-galactosamine | ++ | + | + | 0 | 0 | 0 |
| *N*-Acetyl-D-glucosamine | ++++ | +++ | +++ | ++ | ++ | + |
| D-Glucose | ++++ | ++++ | ++++ | ++ | ++ | 0 |
| D-Galactose | ++++ | +++ | +++ | ++ | ++ | + |
| D-Mannose | ++++ | ++++ | +++ | ++ | ++ | + |
| DL-Fucose | ++++ | ++++ | ++ | + | + | ± |

Table 2. Inhibition of agglutination of human erythrocytes by PHA with *N*-acetyl-D-galactosamine. In each reaction mixture final concentration of sugar was 50 mg/ml. The readings were made 10 minutes after incubation at 37°C.

| Sugar added | Agglutination at dilutions of PHA | | | |
|---|---|---|---|---|
| | 1:250 | 1:500 | 1:750 | 1:1000 |
| None | +++ | +++ | ++ | + |
| *N*-Acetyl-D-galactosamine | + | + | ± | 0 |
| *N*-Acetyl-D-glucosamine | +++ | +++ | + | + |
| D-Galactose | +++ | ++ | +++ | ++ |
| D-Glucose | ++++ | ++ | + | ± |
| L-Fucose | +++ | ++ | + | + |

Incubation of rat lymphocytes or erythrocytes with *N*-acetyl-D-galactosamine (50 mg/ml) for 10 minutes followed by two washings with MEM did not result in a decrease in their agglutinability by PHA. Moreover, PHA incubated with *N*-acetyl-D-galactosamine and then dialyzed against 1000 volumes of MEM for 16 hours agglutinated rat lymphocytes to the same extent as dialyzed samples of PHA which had not been exposed to the sugar. These experiments indicate that the inhibitory effect of *N*-acetyl-D-galactosamine is due neither to the destruction of PHA nor to an irreversible alteration of the cell. Magnesium ion which overcomes the inhibitory action of ethylenediaminetetraacetic acid on PHA hemagglutinating activity (*9*) had no effect on the inhibition by *N*-acetyl-D-galactosamine.

The mechanisms by which PHA's cause agglutination of leukocytes and erythrocytes are unknown. In our study evidence has been obtained that *N*-acetyl-D-galactosamine specifically inhibits hemagglutination and leukoagglutination by PHA without destroying either the cells or the PHA. One explanation for the selective inhibition by *N*-acetyl-D-galactosamine of the interaction between the cell and PHA is that this sugar acts in a manner analogous to a hapten and it might participate in this interaction either as a constituent of the cell surface or of the PHA. Since it is known that *N*-acetyl-D-galactosamine occurs as an externally accessi-