In all the experiments, the tendinous ends of the semitendinosus muscles were tied with silk thread and the resting length between the ends was measured *in situ*. After mounting the muscle fibers at resting length in the chamber, the thread from one of the tendons was fixed to a micromanipulator and the fibers were stretched until the length of a segment in the zone of optical recording was increased by 80 to 100 percent. At this degree of stretch, the light scattering associated with contraction was abolished (*4*). The excitability of the muscle fibers was not significantly affected by this stretch. In preliminary experiments in which hypertonic sucrose-Ringer solution was used as another means of dissociating excitation from contraction results similar to those described below were obtained. Optical signals during excitation could be observed when contraction was present, but elimination of contraction permitted more rapid signal averaging and higher amplification.

By the method described above, reproducible and consistent changes in turbidity, birefringence, and fluorescence (of fibers stained with pyronine B) were found in muscle fibers coincident with the action potential. Record A in Fig. 1 shows that the earliest change in the intensity of light scattered by muscle fibers at 90° (lower trace) is coincident with the externally recorded action potential (upper trace). In order to compare the time course of the electrical and optical signals, intracellular recordings from single muscle fibers in the middle pool (C_1) were made by use of micropipettes filled with KCl. The peaks of the intracellularly recorded action potentials occurred 0.2 to 0.8 msec after the peaks of the extracellularly recorded action potentials, which indicates that the peak of the initial upward deflection of the optical trace roughly coincides with the peak of the internally recorded action potential. (That the optical signal coincident with the action potential is due to an increase in turbidity and not to a change in absorption could be shown by reversal of the optical signal at 0°.) Neither the time course nor the magnitude of the optical signal was found to vary significantly with the wavelengths of the incident light between 450 and 650 nm.

To detect birefringence, a polarizer was positioned below the chamber with the plane of polarization at 45° to the longitudinal axis of the fibers and an analyzer was placed above the muscle in the crossed polar position. Under these conditions, there was a decrease in light intensity concurrent with the action potential (see record B). This decrease in the birefringence of the fibers could be observed with all the wavelengths examined between 450 and 650 nm. No optical signal was obtained with the polarizer and analyzer in the crossed polar position and the plane of polarization either parallel or perpendicular to the long axis of the muscle.

In fluorescence studies, the middle chamber was initially filled with Ringer solution containing 0.05 mg of pyronine B per milliliter. The portion of muscle in the middle pool was stained with dye solution for 10 minutes and then washed with dye-free Ringer. Neither the amplitude nor the duration of the action potential was affected by staining with this fluorochrome. The interference filter (F_1), inserted between the muscle and the light source, had a peak of transmission at a wavelength of 550 nm, which corresponded to the absorption maximum of pyronine B (*5*). A secondary filter (F_2) placed between the muscle and the photomultiplier cut off all light shorter than 610 nm in wavelength. Record C shows a transient decrease in fluorescence beginning at the onset of the action potential.

The following observations show that the fluorescence signal was not due to artifacts such as imperfect transmission characteristics of the filters or the effects of electric current on the dye molecules. (i) If the muscles were not stained, but the rest of the experimental procedure remained unchanged, no optical signal was obtained. (ii) When the cut-off filter (F_2) was positioned between the interference filter and the muscle, no early optical signal was observed. (iii) When the stimulating electrodes were reversed, there was no change in the fluorescence signal after stimulation until the action potential was conducted from the lateral to the middle chamber.

The earliest changes in the optical properties of the muscle fibers described in this report are coincident with the action potential. The variations in light intensity subsequent to the early optical signals in records A, B, and C are most likely due to changes in the optical properties of the fibers during the period of latency relaxation (*4, 6*). The early increase in turbidity, decrease in birefringence, and decrease in fluorescence (of fibers stained with pyronine B) following electrical stimulation of muscle are similar to the changes in the optical properties observed in nerve during excitation (*2, 3*). In other experiments to be detailed elsewhere, similar changes in fluorescence during the action potential of giant barnacle muscle fibers stained with pyronine B, in which excitation was dissociated from contraction by internal injection of ethylenediaminetetraacetate, have been detected. This similarity may be an indication that the excitation process is due to comparable conformational changes in the membrane macromolecules of nerve and muscle.

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Unexpected Adrenergic Effect of Chlorpromazine: Eating Elicited by Injection into Rat Hypothalamus

Abstract. *Although chlorpromazine is believed to block adrenergic transmission, injection of this drug into the hypothalamus of satiated rats does not block norepinephrine-elicited eating, but instead mimics norepinephrine by eliciting eating. The amount of eating elicited by norepinephrine and by chlorpromazine is reliably correlated. These results suggest that endogenous norepinephrine mediates eating elicited by centrally injected chlorpromazine.*

Chlorpromazine (CPZ), a tranquilizing agent, is probably the most widely used drug in the treatment of mental illness. Its mechanism of action on the central nervous system, however, still

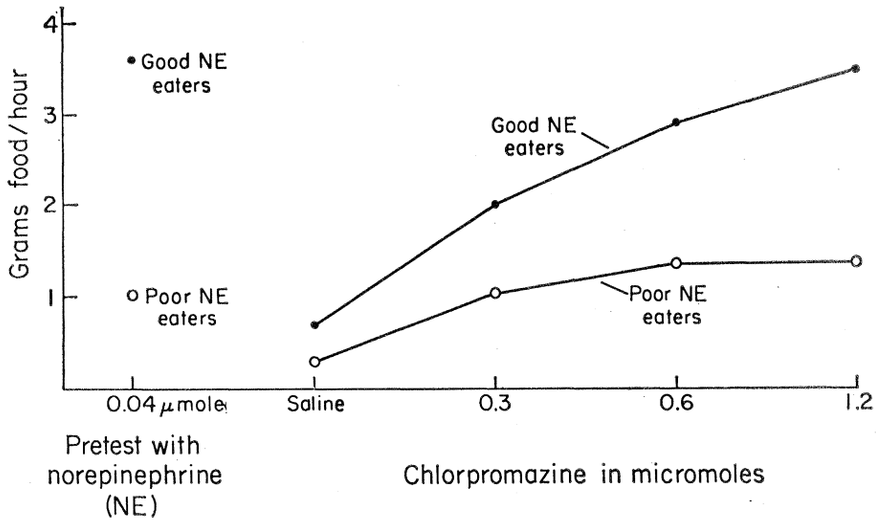

Fig. 1. Mean number of grams eaten per hour by good and by poor NE eaters during the 1st hour after injection of NE and during the first 2 hours after injection of chlorpromazine and of saline.

remains obscure. For a number of years, chlorpromazine has been firmly associated with the adrenergic neurohumoral system on the basis of its α-adrenergic blocking action in the peripheral nervous system (*1*). Recently, several investigators have discovered that CPZ increases the turnover of catecholamines in the brain (*2*). To remain consistent with the traditionally accepted belief in the blocking action of CPZ, these authors have attributed the central activating effects of this drug to a compensatory feedback mechanism after its blockade of the postsynaptic receptors.

As part of a study of the hypothalamic hunger mechanism, we have been following up the finding of Grossman (*3*) that norepinephrine (NE), when injected directly into the hypothalamus via a chronically implanted cannula, elicits eating in rats. If CPZ achieves its clinical effects by blocking adrenergic transmission in the brain, this drug would be expected to block the eating effect elicited by adrenergic stimulation in the hypothalamus. We tested for the central blocking action of CPZ by injecting this drug directly into the hypothalamus shortly before injection of NE. However, we discovered that, in addition to having no apparent effect upon NE-induced eating, CPZ itself elicited eating. Peripheral injections of CPZ have been found to both increase and decrease food intake in human patients and in animals (*4*). The effect of central injection of CPZ upon appetitive behavior has not been examined. Therefore, we decided to investigate this problem with a CPZ dose-response study.

Chronic unilateral cannulas were stereotaxically implanted in the hypothalamus of 44 male albino rats (Sprague-Dawley strain, 300 to 350 g) as described previously (*3*). Histological verification of the intended placements showed that the tips of the cannulas were located in the suprafornical region immediately posterior to the anterior hypothalamus. This region in the hypothalamus has been found to be maximally sensitive to adrenergic stimulation (*5*).

After a 4-day postoperative recovery period, the rats were tested with NE (0.04 μmole) in 1 μl of normal saline and with normal saline alone. The microinjections of NE and normal saline followed a counterbalanced sequence and were at least 2 days apart. All tests started at approximately 9:00 a.m., at which time the rats were given food and water ad libitum in the test cage for 1 hour to ensure maximum satiation. The rats' food and water intake were recorded at the end of the 1st, 2nd, 4th, 8th, and 23rd hours after injection. During the 1st hour after injection, each rat was carefully observed, and all responses (including appetitive and emotional) were recorded.

After completion of the pretests with NE, the rats were separated into two groups, good "NE eaters" ($N = 24$) and poor "NE eaters" ($N = 20$), according to whether they ate more or less than 2.5 g of food after injection of NE. Each rat was then injected with 1 μl of a normal saline solution containing either 0.0, 0.3, 0.6, or 1.2 μmole of CPZ (*6*). These injections, which were always 1 week apart, were counterbalanced in a 4 by 4 Latin square design. The subjects' food and water intake were recorded in the same way as during the pretest with NE. Following the completion of this Latin square, the rats were once again tested with NE (0.04 μmole).

During the 1st hour after injection of normal saline, both the poor and good NE eaters ate less than 0.5 g. During the 1st hour after injection of NE, the poor eaters ate reliably more (1.0 g, $P < .05$) than they did when injected with normal saline, and the good eaters ate reliably more than the poor eaters (3.6 g, $P < .001$). There was essentially no difference in the amount of eating elicited by NE before and after the dose-response tests with CPZ.

Figure 1 shows the CPZ dose-response results, in addition to the NE pretest results. The scores given for food intake following the injection of CPZ or normal saline represent the average amount eaten per hour during the first 2 hours after injection. All three doses of CPZ elicited a reliable increase in food intake in both the poor NE eaters ($P < .01$) and the good NE eaters ($P < .001$). Both groups also exhibited a significant positive relationship between the increase in the dose of CPZ and the increase in food intake ($P < .001$). The amount of eating elicited by CPZ, however, was reliably greater for the good NE eaters than it was for the poor NE eaters ($P < .001$). Furthermore, the magnitudes of the eating responses elicited by NE and by CPZ in all rats were found to be reliably correlated ($r = +.62$, $P < .001$). These results clearly demonstrate that CPZ and NE have similar, rather than antagonistic, effects when injected directly into the hypothalamus.

Chlorpromazine elicited eating to a significant extent during the 2nd hour after injection and to a progressively lesser extent during the 3rd to 8th hours. This longer-lasting effect contrasts with the effect of NE on eating, which lasted for only 30 minutes. For the good NE eaters, the mean latency of the eating response after injection of CPZ was 25 minutes, whereas the mean response latency after injection of NE was 4 minutes.

Chlorpromazine produced several emotional effects, such as sedation, ptosis, and increased aggressive behavior (biting and attacking). These

changes in emotional behavior became most apparent within a few minutes after injection of the stronger doses of CPZ, and they gradually disappeared during the next few hours. Chlorpromazine had no apparent effect upon water intake. Norepinephrine also failed to alter water intake to a measurable extent, although 45 percent of the rats injected with NE (and none of those injected with CPZ) drank for a few seconds before starting to eat.

Additional investigations with CPZ, in which we examined the interaction between pretreatment with CPZ and subsequent hypothalamic injection of NE, further demonstrate the failure of CPZ to interfere in any way with the eating induced by centrally-injected NE (*7*). These experiments tested the effects upon NE-induced eating of CPZ in a single hypothalamic injection, a single intraperitoneal injection, and a series of daily intraperitoneal injections over a period of at least 1 month. In none of these experiments was CPZ found to have any blocking effect upon the amount of food eaten after hypothalamic injection of NE. In these experiments, NE was injected into the rats pretreated with CPZ, always at a time when they were not eating to CPZ, but when the emotional effects of CPZ, such as increased aggression to central injection and sedation and ptosis to intraperitoneal injection, were clearly evident. The presence of these emotional effects was interpreted as an indication that CPZ was pharmacologically active in the brain when NE was injected. These results therefore suggest that, in the "adrenergic feeding" system of the brain, CPZ does not block the postsynaptic receptors, contrary to what would be expected on the basis of its peripheral actions.

Instead, all of our findings suggest a quite different hypothesis: that the central effects of hypothalamically injected CPZ are mediated by endogenous NE, and that a possible mode of action is a CPZ-induced increase in turnover of endogenous NE (*2*). This hypothesis would explain our findings that CPZ, like NE, elicits eating when injected directly into the adrenergic feeding center of the hypothalamus and that the magnitude of this CPZ effect is positively correlated with the magnitude of the eating response elicited by NE. This hypothesis would also predict that hypothalamic pretreatment with NE-depleting drugs should reduce eating induced by CPZ, but not that induced by NE. We have recently performed this experiment and have confirmed this prediction (*7*).

Since Bradley *et al.* (*8*) found that CPZ has an effect similar to that of NE only on neurones that were inhibited by NE (it blocked neurones excited by NE), our results may be interpreted as suggesting that, instead of eliciting eating directly by excitation, CPZ (and NE) elicit eating by inhibiting the hypothalamic system (possibly involving the ventromedial nucleus) which normally suppresses eating.

The classical view has been that CPZ is pharmacologically an adrenergic blocking agent and clinically a tranquilizer. These effects are supposed to be opposite to those of imipramine, a potentiator of adrenergic effects and an antidepressant. However, these two drugs are chemically similar, and CPZ and other phenothiazines have been found to be effective, like imipramine, in treating certain patients with depressions (*9*). It is suggested here that the unique clinical effectiveness of CPZ can be attributed to the complexity of its action, part of which is the central adrenergic effect demonstrated in the present study (*10*).

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10. This hypothesis does not rule out the possibility that CPZ may derive some of its clinical effects from an adrenergic blocking effect on peripheral, or even on certain central, neurones. A few investigators have suggested that CPZ, at least in the peripheral nervous system, inhibits the uptake of NE into the nerve endings, in a fashion similar to that of imipramine-like drugs [(*1*) and J. Häggendal and B. Hamberger, *Acta Physiol. Scand.* **70**, 277 (1967)]. However, since hypothalamic injection of the potent uptake inhibitor, desmethylimipramine, fails to elicit the consistent eating elicited by CPZ (our unpublished data), we feel that an effect on uptake cannot be the primary explanation of the eating effect of CPZ. Instead, we feel that this adrenergic action of CPZ in the hypothalamus probably results from its effect of accelerating the synthesis and release of catecholamines (*2*).
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Hurler's Syndrome: Deficiency of a Specific Beta Galactosidase Isoenzyme

Abstract. *A marked deficiency of a specific thermolabile β-galactosidase isoenzyme (*p*H optimum 3 to 5) was found in liver and kidney tissues of five patients with the Hurler's syndrome (types 1 to 3).*

The Hurler's syndrome is a genetically transmitted disorder of mucopolysaccharide metabolism characterized by excessive visceral storage of chondroitin sulfate B and heparitin sulfate (*1*) and excessive cerebral storage of gangliosides GM_1, GM_2, and GM_3 (*2*). At least six phenotypically distinct types of the Hurler's syndrome have been delineated (*1*). The fundamental enzymic defect in each type is unknown. One possibility is a deficiency of a hydrolytic enzyme which participates in the cleavage both of mucopolysaccharides and glycolipids. This possibility is enhanced by evidence from (i) morphological studies indicating that the storage substances are in lysosomes (*3*), (ii) kinetic studies which demonstrate an impairment in the deg-

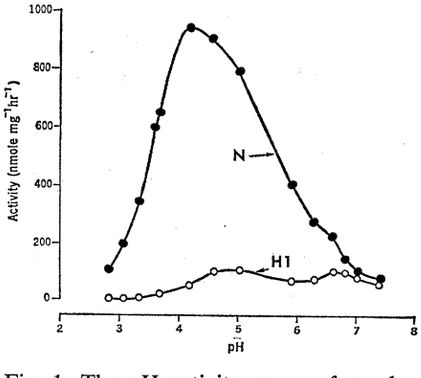

Fig. 1. The *p*H activity curve of β-galactosidase from normal (*N*) and Hurler's type 1 (*H1*) liver. Activity was determined with *p*-nitrophenyl galactoside as substrate ($10^{-3}M$) in citrate-phosphate buffer ($0.1M$) and is expressed as nmoles of *p*-nitrophenyl galactose cleaved per milligram of protein per hour at 37°C.