stricted set of genes than do the HMG proteins.

Previous efforts to correlate the subnuclear localization of the T_3 receptor with nuclear functions have yielded conflicting results. Charles *et al.* (*13*) first demonstrated an enrichment of the receptor in slowly sedimenting sheared rat liver and HeLa cell chromatin containing high RNA polymerase II activity, and suggested on the basis of this observation that the T_3 nuclear receptor was associated with transcriptionally active chromatin. Subsequent reports from this laboratory (*14*), however, have indicated a failure to obtain an enrichment of the T_3 receptor in transcriptionally active chromatin in pituitary tumor cells of the GH line by means of DNase II. In contrast, Samuels *et al.* (*15*), using DNase II effected a threefold enrichment of the receptor in transcriptionally active chromatin from GH_1 pituitary cell nuclei. The basis for these discrepancies is not apparent to us.

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19. After submission of this manuscript, Samuels *et al.* [H. H. Samuels, F. Stanley, W. Casanova, T. C. Shau, *J. Biol. Chem.* **255**, 2499 (1980)] demonstrated that micrococcal nuclease digestion of rat pituitary GH_1 cell nuclei excises the T_3 receptor as a predominant 6.5*S* form. These authors also reported that a less abundant 12.5*S* form of receptor is released from chromatin by nuclease. We have observed that, under velocity sedimentation conditions different from those described herein, micrococcal nuclease liberates the T_3 receptor as a predominant 5.8*S* form and minor 12*S* to 12.5*S* form from euthyroid rat liver nuclei.
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Evidence for L-Glutamate as the Neurotransmitter of Baroreceptor Afferent Nerve Fibers

Abstract. *Microinjection of L-glutamate into the intermediate nucleus tractus solitarii in anesthetized rats elicits hypotension, bradycardia, and apnea, simulating baroreceptor reflexes. Ablation of the nodose ganglion results in selective reduction of high-affinity uptake of L-glutanate in the nucleus tractus solitarii. L-Glutamate may be the neurotransmitter of afferent nerve fibers from arterial baroreceptors.*

Primary afferent fibers of arterial baroreceptors projecting through the ninth and tenth cranial nerves terminate in the medulla oblongata on intrinsic neurons within the middle third of the nucleus tractus solitarii (NTS) (*1*). These neurons relay signals from baroreceptors to other areas of brainstem and spinal cord, thereby eliciting hypotension, bradycardia, and apnea, the cardinal responses to baroreceptor stimulation (*2*).

The baroreflex may be modulated centrally by neurotransmitters of several classes, including the catecholamines norepinephrine and epinephrine (*3*), γ-aminobutyric acid (GABA) (*4*), and several neuropeptides (*5*). These transmitters, however, seem to be contained in processes of neurons whose cell bodies reside within the central nervous system (CNS). The identity of the neurotransmitter synthesized, stored, and then released into NTS by the primary baroreceptor afferents is unknown.

We have examined the possibility that the amino acid L-glutamate, a putative transmitter in several intrinsic pathways of the CNS (*6*) and of dorsal root afferent fibers (*7*), may be the neurotransmitter released from baroreceptor afferents within the NTS.

We first examined the physiological effects of microinjecting L-glutamate into the NTS. Rats were anesthetized with halothane, and a cannula was inserted into the aorta through the ventral tail artery for recording arterial pressure and heart rate. The animals were placed in a stereotaxic frame, and the dorsum of the medulla was exposed. Drugs were dissolved in 0.9 percent saline and injected directly into the NTS through a glass micropipette with a tip 50 to 100 μm in outside diameter. Exactly 0.1 μl of each substance was injected in 1 second by a microinfusion pump. In some rats, the location of the cannula was verified by injecting drugs mixed with methylene blue. The dye did not alter the response to the injected agent. After the experiment, the animals were killed, the brains sectioned, and the injection sites identified.

L-Glutamate injected unilaterally resulted, after a latency of 1 to 5 seconds, in the development of hypotension, bradycardia, and apnea (Fig. 1A). The cardiovascular responses depended on dosage (Fig. 1B), appearing at a dose of 5 ng (30 pmole) and reaching a maximum at approximately 1000 ng. The average response in eight animals after a unilateral injection of 1000 ng was to lower mean arterial pressure by 37 ± 3.5 mm-Hg from a baseline level of 84 ± 2.0 mm-Hg and to slow the heart rate by 53 ± 10.9 beats per minute from a baseline level of 344 ± 21.4 beats per minute. Saline (0.9 percent) injected into the NTS usually had no cardiovascular effect, although the average effect in 32 rats was a fall of arterial pressure by 5 ± 1.2 mm-Hg and of heart rate by 6 ± 1.9 beats per minute. Within a dosage range of 5 to 100 ng, the responses to L-glutamate could be elicited with each subsequent injection. At higher doses (> 500 ng), they could not be repeated for at least 30 minutes after injection.

The hypotensive effects of L-glutamate were anatomically specific and restricted to the intermediate third of the NTS, as verified histologically. Injections of L-glutamate as high as 100 ng into sites adjacent to the NTS (including the area postrema, median raphe nuclei, and external cuneate nucleus) and into the dorsal medullary tegmentum failed to elicit any cardiovascular responses. Injection of the L-glutamate analog kainic acid

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Table 1. Specific uptake (in picomoles per milligram of protein per minute) of 1 μM L-[^{3}H]glutamate or [^{3}H]GABA by rats 14 days after removal of the right nodose ganglion. Values are expressed as means ± standard errors of the mean. The number of animals in each group is given in parentheses.

Substance	Control*	Lesion	
		Right	Left
	Nucleus tractus solitarii		
L-[^{3}H]Glutamate	103 ± 6.7 (12)	67 ± 3.5 (6)†	65 ± 2.4 (6)†
GABA	99 ± 13.0 (6)	103 ± 23.0 (6)	
	*Spinal trigeminal tract**		
L-[^{3}H]Glutamate	51 ± 10.2 (5)	50 ± 11.1 (5)	

*Tissues from right and left sides were pooled. †Comparisons are with control data; t (16) = 3.016, $P < .01$.

(KA) into the NTS also resulted in hypotension, bradycardia, and apnea. The threshold for KA was 0.2 ng (1 pmole), a dose substantially lower than that required for L-glutamate. Kainic acid in doses greater than 6 ng blocks baroreflexes and the evoked response in the NTS to electrical stimulation of aortic depressor or vagal nerves and, after termination of anesthesia, results in fulminating hypertension (*8*). GABA (100 ng) microinjected into the NTS had no effect on arterial pressure or heart rate.

The nodose ganglion contains the cell bodies of many of the arterial baroreceptors, and its removal results in intense degeneration of the NTS caudal to the obex (*9*). To determine whether fibers projecting into the NTS via the visceral afferent nerves were mediated by glutamate, we examined the effects of extirpation of the right nodose ganglion on the high-affinity uptake of L-glutamate into nerve endings within the NTS.

For one group of rats, the right nodose ganglion was excised (*10*); age-matched rats, without the operation, served as controls. Two weeks later the animals were killed, the brainstems removed, and small samples taken from the ipsi- and contralateral third of the NTS and, as a control, from the spinal trigeminal tract. Tissues were homogenized and the uptake of [^{3}H]glutamate and also [^{3}H]-GABA were measured in these homogenates (*11-13*).

Two weeks after the removal of the right nodose ganglion, the high-affinity uptake of L-glutamate was reduced 40 percent in both ipsi- and contralateral NTS (Table 1). There was no concomitant change in the uptake of 1 μM GABA into the NTS or of L-glutamate in the spinal trigeminal tract.

Locally injecting very low doses of L-glutamate or its analog KA into the NTS thus resulted in a dose-dependent reduction of arterial pressure and heart rate and also apnea, thereby simulating the baroreceptor reflex. The responses were anatomically restricted to the intermediate third of the NTS—the site of termination of baroreceptor afferent fibers—and were not elicited by GABA. Since microiontophoresis of L-glutamate will excite most neurons in the CNS (*14*), the cardiovascular effects of L-glutamate might result from the production of responses comparable to those elicited by focal electrical stimulation (*15*), a nonspecific excitation of local neurons. However, the finding of a substantial reduction of the high-affinity uptake of L-glutamate largely confined to the intermediate third of the NTS after extirpation of the nodose ganglion suggests that many of the fibers projecting into the region store and release L-glutamate. Indeed, a decrease in the high-affinity uptake for L-glutamate in a terminal region after lesions are made in a projection pathway is generally considered the most sensitive biochemical index that such a pathway is glutamatergic (*13*).

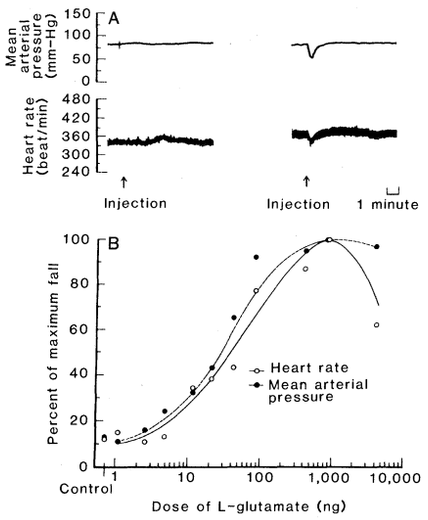

Fig. 1. (A) Hypotension and bradycardia elicited by microinjection of L-glutamate (right) but not saline (left). (B) Dose-response curves normalized to a maximum fall of 37 ± 3.5 mm-Hg for arterial pressure and of 53 ± 10.9 beats per minute for heart rate. Each point on the dose-response curve represents the data from one injections of each dose in each of eight separate animals. Control injections of saline caused arterial pressure to fall by 5 ± 1.2 mm-Hg and heart rate by 6 ± 1.9 beats per minute, which did not significantly differ from the response to the lowest doses of L-glutamate.

The decreased uptake of L-glutamate in the NTS produced by unilateral ablation of the nodose ganglion and its bilaterality were surprising. Relatively few fibers in the nodose ganglion arise from arterial baroreceptors, compared with those arising from other visceral afferents (*16*). The results therefore suggest that either (i) L-glutamate is highly concentrated in those fibers arising from cardiopulmonary receptors or (ii) it is also contained in other afferent nerve fibers (including those from the lung and the gut) that may also reflexly initiate autonomic responses comparable to those of the baroreceptors (*17*). While it is known that some fibers of the ninth and tenth nerves are crossed (*9*), these are considered rare. The size of the contralateral decrease in L-glutamate after nodose ganglionectomy suggests that most decussating fibers contain L-glutamate.

The demonstration that microinjecting L-glutamate into the NTS simulates baroreflexes and that ablation of afferent fibers from baroreceptors into the region reduces the uptake of L-glutamate supports the hypothesis that this amino acid may be the neurotransmitter released by baroreceptors within the region. Such information may help not only to further delineate the role of L-glutamate in central neurotransmission but also to provide new neurochemical approaches to hypertension and to the development of drugs used in the treatment of this disease.

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11. The rats were decapitated, and the brainstems were removed and placed on a glass plate over ice. A 1-mm transverse slice was removed with the caudal edge of the slice at the calamus scriptorius. Regions were removed from the fresh unfrozen slice with a microbore punch (inside diameter, 1 mm), by the method of Palkovits (*12*), and were homogenized in 0.32*M* sucrose. The homogenate was centrifuged for 10 minutes at 1000*g*. The high-affinity uptake of 1.0 μM [^{3}H]glutamate or 1.0 μM [^{3}H]GABA was determined (*13*) in the homogenate containing 5 to 15 μg of protein for 1 minute at 30°C in 250 μl of freshly oxygenated buffer (Krebs-Phosphate) whose [Ca^{2+}] was halved to 1.2 m*M*. Uptake was terminated by vacuum filtration through filters (GF/B). Nonspecific uptake was determined in parallel incubations in Na^+-free medium (osmolarity maintained with sucrose). Separate experiments showed that uptake was linear with protein concentration under these conditions.
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Histamine-Mediated Delayed Permeability Response After Scald Burn Inhibited by Cimetidine or Cold-Water Treatment

Abstract. *Scald injury to one ear of the hairless mouse induced significant ($P < .05$) delayed edema formation in remote, uninjured skin. This remote edema formation was completely inhibited by immediate cold-water treatment of the scalded ear. Cold-water treatment significantly reduced histamine loss from the scalded ear, and the edema-inhibiting effect of the treatment could be mimicked by treating the animal prior to injury with the H_2-histamine receptor antagonist cimetidine or a drug that causes histamine depletion. These observations suggest (i) that a histamine-mediated, delayed permeability response occurs after thermal injury that causes remote edema formation and (ii) that one mechanism of remote edema inhibition by cold-water treatment is the prevention of histamine release from thermally injured tissues.*

Scald injury (54°C water for 20 seconds) to a single ear of the hairless mouse significantly ($P < .05$) increased the water content of the skin (edema) of the uninjured ear and the abdomen for up to 2 to 6 hours. This remote edema formation was completely abolished after immediate cold-water treatment (CWT) of the burned ear for 5 minutes in 8° to 10°C water. Chemical reproduction of this effect could be achieved by treating the animals prior to injury with cimetidine, or a drug that causes histamine depletion. Tissue histamine loss from ears subjected to scald injury and CWT was significantly decreased when compared to untreated burns. Ultrastructural features of the microvasculature in uninjured skin displaying increased water content at 2 hours after injury included the presence of endothelial vacuoles thought to be associated with increased permeability. These findings suggest that a histamine-mediated, delayed systemic permeability response occurs after scald injury, and that this response causes remote edema formation that can be chemically inhibited with the H_2-histamine receptor antagonist cimetidine. It has been generally accepted that after mild or moderate thermal injury the mediated effects of histamine are limited to "immediate, transient" local permeability responses of the burn wound not observed beyond 0.5 hour after injury (*1*). Our study suggests that histamine may also mediate delayed increases in vascular permeability. In addition, we verify the phenomenon of remote burn edema and suggest that burn wound histamine release is related to both postburn hypovolemia and the protective influence of CWT in preventing burn shock.

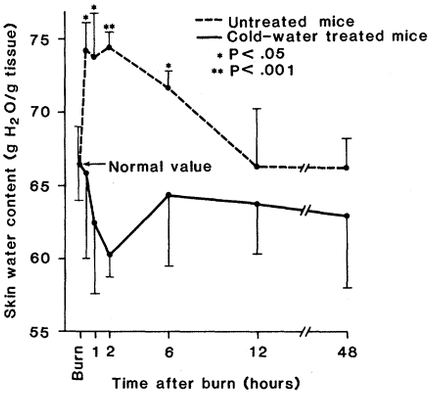

Fig. 1. Comparison of remote edema formation in the unburned ears of burned mice versus burned mice that received CWT for 5 minutes. Cold-water treatment of the burned ear completely abolished remote edema formation in the unburned ear. The skin water content of the unburned ears of animals that received CWT as depicted here, is not significantly different from time-matched controls that received only anesthesia. The *P* values compare the water content of skin from unburned ears of mice that were given CWT. Each data point represents six animals.

For experimental observations we used male, homozygous (*hr/hr*) hairless mice (Jackson Laboratories; 28 to 35 days old) anesthetized with Diabutol (50 mg/kg). Their left ears were exposed to 54°C water for 20 seconds. This resulted in a reproducible burn injury covering about 6 percent of the total body surface area (*2*). To determine the extent of edema in the contralateral, unburned ears of the injured mice we measured the difference between the wet weight and dry weight of the skin (Fig. 1). In the unburned right ear the skin water content was increased by 12 percent (maximum value) at 0.5 and 2 hours after burn injury of the left ear. The increased skin water content of the unburned ears was significant between 0.5 and 6 hours after injury ($P < .05$, Student's *t*-test). At 6 hours after injury to the left ear the edema in the unburned ear began to decrease gradually, with the water content approaching normal values by 9 hours. Mouse abdominal skin obtained at 2 hours after scald injury to the ear showed a similar increase in water content. Such increases in the skin water content at uninjured remote sites following moderate scald injury has not to our knowledge been reported previously.

We also conducted morphological studies of unburned ear skin which demonstrated significant increases in water content after the opposite ear had received a burn injury. Ultrastructurally, the skin of these unburned ears displayed two changes that may be related to increased vascular permeability. The intercellular clefts of capillaries and small venules sometimes contained small dilatations (but not complete patencies in any single section) similar to those seen by Casley-Smith and Window (*3*) in directly burned sites or after application of histamine. Also, in capillaries and, especially, venules, we observed numerous small endothelial vacuoles. These vacuoles were always distinctly larger than micropinocytotic vesicles and, when attached either to the luminal or abluminal cellular membranes, they were closed by a diaphragm (Fig. 2). We studied similar specimens from the ears of mice injected intravenously with colloidal carbon (~250 to 300 Å) and were unable to demonstrate vacuolar inclusion of carbon; however, transport of ferritin (~110 Å) in ultrastructurally similar vacuoles

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