Microelectrophoresis of Biogenic Amines on Hypothalamic Thermosensitive Cells

Abstract. *The rat hypothalamus contains thermally insensitive, normally sensitive, and highly thermosensitive cells. The responses of thermosensitive neurons to microelectrophoretically applied acetylcholine, norepinephrine, and 5-hydroxytryptamine were the same in both rats and cats. The firing rate of warm-sensitive interneurons was accelerated by acetylcholine and inhibited by norepinephrine. The firing rate of cool-sensitive interneurons was accelerated by norepinephrine and, in one case, was inhibited by 5-hydroxytryptamine. Thermodetector cells were relatively insensitive to these amines, but were sensitive to current flow. These results from the rat, but not from the cat, agree with the data for thermoregulatory responses following microinjection of these amines into the hypothalamus.*

Microinjection of norepinephrine (NE), 5-hydroxytryptamine (5-HT), and acetylcholine (ACh) into the preoptic area and anterior region of the hypothalamus (PO/AH) or the adjacent ventricle produces changes in the body temperature of several species of animals (*1*). In the rat, NE has been shown to produce an increase in behavioral and physiological heat-production responses, resulting in increased hypothalamic temperature (T_{hy}) (*2*). Acetylcholine, by contrast, activated behavioral and physiological heat-dissipation responses, resulting in decreased T_{hy} (*3*). Similarly, 5-HT has been shown to produce a decrease in rectal temperature (T_r) (*4*). Experiments in cats have shown that microinjection of NE produces a decrease, whereas 5-HT produces a rise, in T_r (*5*). These observations raise the question as to the effects of these endogenously found compounds (*6*) on the activity of hypothalamic neurons. The purpose of this investigation was to test the assumption that these amines produce a thermoregulatory response by changing the rate of firing of temperature-sensitive cells located within the PO/AH. Our data support this assumption and, further, demonstrate functional differences between various types of temperature-sensitive cells.

The present experiments were conducted in 16 male Sprague-Dawley rats and 18 cats of both sexes. The animals were anesthetized with urethane (1 g/kg, intraperitoneally) and placed in a stereotaxic instrument. Rectal temperature was continuously monitored with a thermistor probe, and was maintained at 37.5° to 38°C by means of a heating pad. A bead thermistor, placed symmetrical with and contralateral to the PO/AH recording site, was used to sense T_{hy}. Two water-perfused thermodes (*7*) were used to change T_{hy} in the rat, placed bilaterally 2 mm lateral of the midline and 2.5 mm anterior to the PO/AH. Four thermodes, placed bilaterally 4.5 mm from the midline and with an anterior-posterior separation of 8 mm, bracketed the PO/AH in the cat. Five-barrel glass micropipettes with overall tip diameters of 3 to 6 μm were used to record single cell discharges and to apply various compounds iontophoretically. The recording barrel was filled with 4*M* NaCl. A second barrel was filled with 0.5*M* NaCl and was used to test the effect of current flow on the rate of neural spike discharges. The remaining barrels contained fresh aqueous solutions of 0.25*M* acetylcholine chloride, 0.25*M* norepinephrine bitartrate, and 0.02*M* 5-hydroxytryptamine creatinine sulfate. The micropipettes were filled by combined capillary action (*8*) and centrifugation just prior to each experiment. Cell spike discharges were monitored on the upper beam of a Tektronix RM 565 oscilloscope. The intensity of iontophoretic current applied through the micropipette was monitored on the lower beam. An intensity of 100×10^{-9} amp (100 na) was regularly used to eject the amines and to test for the effects of current flow. Intensities of 5 to 200 na were used occasionally. Ejecting currents were anodal (that is, micropipette positive) since the amines were positive ions. Retaining currents up to -30 na were used when necessary to prevent diffusion of amines out of the micropipettes. However, retaining current was not normally needed because of the low concentrations of the drug solutions. In addition, spike discharges were fed through a level discriminator to a digital rate meter, for determination of the number of firings per second.

Direct plots of either firing rate versus T_{hy} or firing rate versus time were made on an X-Y plotter. Thermal sensitivity of recorded cells was determined from the plots of firing rate versus T_{hy}, as in previous work (*9*). Three types of temperature-sensitive cells were identified, based on consideration of the shape of the curve relating cell firing rate to T_{hy}. The first type was warm-sensitive, responding to increasing T_{hy} with an expo-

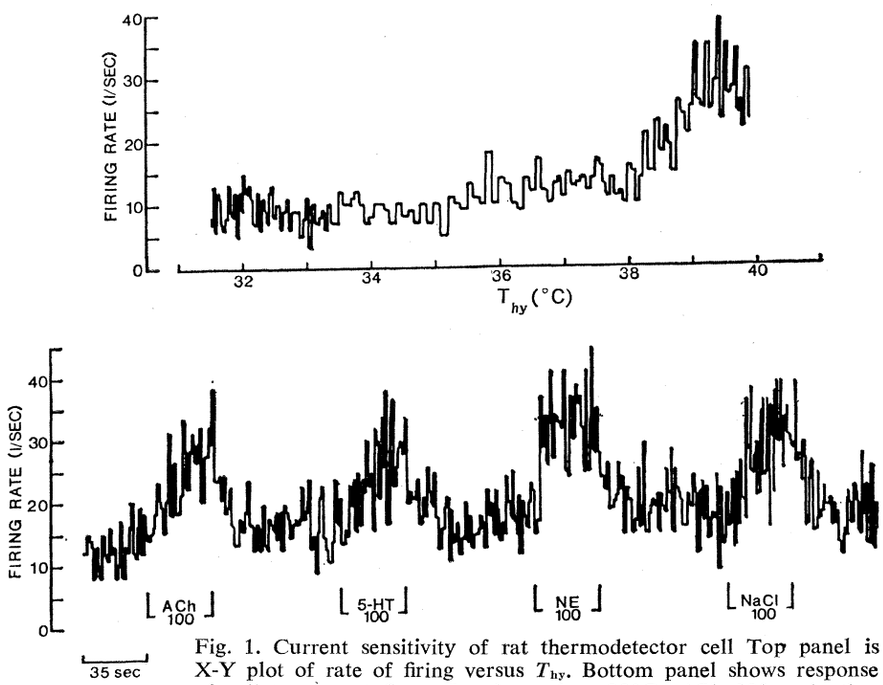

Fig. 1. Current sensitivity of rat thermodetector cell Top panel is X-Y plot of rate of firing versus T_{hy}. Bottom panel shows response of cell to current flow; T_{hy} clamped at 38°C. *ACh 100* and other labels indicate 100 na flowing through indicated barrel.

nential increase in firing rate. Evidence from work in cats (*9, 10*) suggests that these are thermodetector cells. The second type of warm-sensitive cell responded to increasing T_{hy} with increasing firing rate only above a given value of T_{hy}. A third type of cell was cool-sensitive, responding to increasing T_{hy} with a decrease in firing rate. These latter two types of neurons are considered to be interneurons in the thermoregulatory effector pathway (*9*). The animals were killed at the conclusion of the experiments by perfusion with 0.9 percent NaCl followed by 10 percent formalin. Frozen sections through the PO/AH were cut at 80 μm and stained with neutral red for determination of the location of the micropipette tracts.

Seventy-three cells were studied in these experiments, 23 from rats and 50 from cats. The present experiments are the first direct recording of thermosensitive cells to be reported for the rat, and show that hypothalamic thermosensitive cells of rats are similar to those found in cats (*9, 11*) and other species (*12*). Three types of temperature-sensitive cells were identified in rats. Seven thermodetector cells (31 percent of the rat sample), responding to increasing T_{hy} with an exponential increase in firing rate, were found. One warm-sensitive interneuron (4 percent), responding to increasing T_{hy} with a decrease in firing rate, was recorded. Thirteen thermally insensitive cells (56 percent) as well as one normally thermal sensitive cell (4 percent) were also found. A higher percentage distribution of thermodetectors was found in rats as compared with other species (*9, 11, 12*), but this might be due to the relatively small size of the population of rat cells tested.

Thermodetectors, warm- and cool-sensitive interneurons, and temperature-insensitive cells responded differently to ACh, NE, and 5-HT. Local application of electrical current also tended to affect these cell types differently. The responses of these cell types were the same in both rats and cats. In general, thermodetectors in rats and cats seldom responded to amine application, but often responded to current flow. A total of 19 cells were tested with anodal current. Eight showed a decrease and one an increase in firing rate. Norepinephrine slowed the firing rate of 2 out of 23 cells, 5-HT was without effect in 21 cells, and ACh increased the firing rate in 1 of 24 cells tested. Figure 1 illustrates the current sensitivity of a rat thermodetector. Application of ACh, 5-HT, and NE produced an increase in firing rate which was equal to that produced by the passage of current alone. Thus, the response of the cell was produced by the effects of current flow, and not by an action of the amines.

Table 1. Responses of nonthermosensitive ($Q_{10}1$), normally thermosensitive ($Q_{10}2$), and highly thermosensitive detector-type ($Q_{10}>2$) and interneuron-type cells from rats and cats to NE, 5-HT, and ACh. Numbers are combined data from rats and cats, except numbers in parentheses, which are data from rat cells alone. Not all cells of a given type were tested on all three amines. *N*, total number of rat and cat cells of a given type tested; Inc., increase in firing rate; Dec., decrease in firing rate; N.C., no change in firing rate.

Cell type	*N*	NE			5-HT			ACh		
		Inc.	Dec.	N.C.	Inc.	Dec.	N.C.	Inc.	Dec.	N.C.
$Q_{10}1$ and 2	34 (14)	0	5 (3)	25 (7)	0	2 (1)	27 (9)	(1)	0	31 (11)
$Q_{10}>2$ (detector)	24 (7)	0	2 (1)	21 (5)	0	0	21 (5)	(1)	0	23 (6)
Warm-sensitive interneuron	9 (1)	0	7 (1)	2 (0)	0	0	7 (0)	3 (1)	0	6 (0)
Cool-sensitive interneuron	5 (0)	4	0	1	0	1	3	0	0	5

Warm-sensitive and cool-sensitive interneurons in rats and cats were responsive to amine application, but not, in general, to current flow. Warm-sensitive cells showed increased firing during application of ACh in three out of nine cells tested, and decreased firing with application of NE in seven out of nine cells. Eight cells were tested with anodal current. One responded with a decrease in firing rate and seven remained unchanged. The firing rate of cool-sensitive interneurons decreased during application of 5-HT in one cell out of four tested and increased during the iontophoresis of NE in four of the five cells that were tested. Five cells were tested with anodal current, and all were unaffected. The effect of ACh and NE on a rat warm-sensitive interneuron is illustrated in Fig. 2.

Acetylcholine produced a sharp rise in firing rate, with the effect diminishing during continued application. The attenuation of the response shown here is not a characteristic feature of the action of ACh on this type of cell. Norepinephrine produced a decrease in the firing rate, which occurred in this particular cell after a considerable latency. Current applied in a magnitude equal to that used to deliver the amines was without effect on the firing rate. Thermally insensitive ($Q_{10}1$) and normally sensitive ($Q_{10}2$) cells were inhibited by NE in 5 of 30 cells examined and by 5-HT in 2 of 29 cells. One cell out of 32 tested was excited by ACh. Current sensitivity was seldom observed. Nineteen out of 22 cells tested were unaffected by the application of anodal current. The data relating the amine sensitivity of the cell types examined in this study are presented in Table 1.

Previous investigations have demonstrated certain functional differences between the detector and interneuron type of thermosensitive cells, in addition to the differences in the shape of the

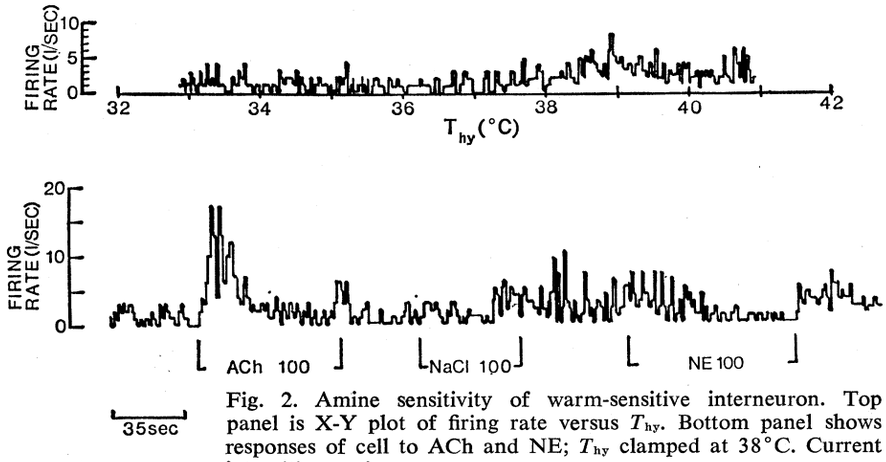

Fig. 2. Amine sensitivity of warm-sensitive interneuron. Top panel is X-Y plot of firing rate versus T_{hy}. Bottom panel shows responses of cell to ACh and NE; T_{hy} clamped at 38°C. Current intensities are in nanoamperes.

thermal response curves. These were related to the types of responses observed following administration of barbiturate anesthesia (*9*) and of pyrogen (*10*). The present work extends the inventory of these characteristics to include differential responsiveness to the passage of current and to the amines tested. Thermodetectors appear to be relatively insensitive to NE, 5-HT, and ACh, but are sensitive to current flow. Thermosensitive interneurons, by contrast, are amine sensitive but relatively insensitive to current. The insensitivity of the thermodetectors to amines suggests a lack of synaptic input to these unique cells.

The responses of the rat's thermosensitive interneurons to ACh and NE shown in this study are in agreement with what would be predicted from the results of previous microinjection studies. That is, microinjection of ACh into the PO/AH produced coordinated behavioral and physiological heat-dissipation responses (*3*). When applied to single cells, ACh increased the firing rate of warm-sensitive cells, an event which would be expected to activate heat-loss responses. Microinjection of NE into the same hypothalamic region results in the activation of coordinated behavioral and physiological heat-production responses (*2*). As shown here, NE decreased the firing rate of warm-sensitive neurons, an effect which would be expected to initiate increases in heat production and conservation responses.

The responses of thermosensitive neurons in the cat to ACh, 5-HT, and NE were the same as those observed in the rat. These data from the cat are thus in disagreement with the results of microinjection experiments. Norepinephrine has been reported to produce a decrease in body temperature when microinjected into the PO/AH (*5*). However, NE depresses the firing rate of warm-sensitive cells and increases the firing rate of cool-sensitive cells. The responses obtained at the single cell level would require NE to produce a rise, rather than a fall, in body temperature. The factors responsible for the discrepancy between the effects of NE recorded at the single cell level and those at the gross physiological level are not immediately apparent.

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Estrogenic Induction of Ornithine Decarboxylase in vivo and in vitro

Abstract. *Injection of estrogens (17β-estradiol or diethylstilbestrol) into immature chicks results in a marked (30- to 50-fold) increase in the ornithine decarboxylase activity of oviductal homogenates within a 4-hour period. Similar stimulations were obtained when estrogen was injected into hypophysectomized or castrated rats and the uterus was examined for decarboxylase activity. An elevation of decarboxylase activity was obtained in vitro when oviducts from immature chicks were incubated in the presence of estrogen. These data indicate a direct action of estrogen on oviduct tissue to promote a rapid increase in the activity of a specific enzyme and represent the first example of a completely in vitro enzyme response to estrogen.*

Rapid induction of ornithine decarboxylase activity in liver after partial hepatectomy or administration of growth hormones has been reported (*1*). We have previously reported (*2*) that epidermal growth factor (a 6400-molecular-weight polypeptide isolated from the submaxillary gland of the mouse) induces a marked, but transient, increase of ornithine decarboxylase activity. This effect is demonstrable both in cultures of chick embryo epidermis and in the skin of mice after injection of this polypeptide. The similarities in the biochemical response of these cell types to a growth-promoting stimulus suggested the possibility that an increase in ornithine decarboxylase activity and subsequent putrescine and polyamine accumulation may be an early event in tissues in which growth is induced. If this hypothesis was both correct and general, then a tissue growth response mediated by a steroid hormone might also be preceded by an early stimulation of ornithine decarboxylase.

The chick oviduct represents a suitable model in which to test this hypothesis (*3*). Estrogen administration to the immature chick induces a marked growth response (1000-fold increase in wet weight) and results in the differentiation of the immature oviduct epithelium into three completely new cell types (*4*). This hormonal response is accompanied by changes in cell RNA populations, the induction of synthesis of a new complement of cell-specific proteins, and the appearance of new tissue functions (*4*).

In the first series of experiments, estrogen was injected either subcutaneously or intravenously into immature 6-day-old chicks. At intervals thereafter, the animals were killed and homogenates of the oviduct were prepared in 0.05*M* phosphate buffer, *p*H 6.6, containing 2 m*M* ethylenediaminetetraacetate (EDTA). The homogenates were centrifuged for 15 minutes at 10,000*g*, and the supernatant fluid was used for the assay of ornithine decarboxylase activity. The enzyme activity was determined by measurement of the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine (*2*).

The results (Table 1) of two such experiments show that 4 hours after the subcutaneous injection of diethylstilbestrol the specific activity of the decarboxylase had increased approximately 50-fold (experiment 1). Following this initial peak at 4 hours, the specific activity of the enzyme gradually diminished over the ensuing 6-day period. When a nonlethal dose of cycloheximide (1 mg) was injected 1 hour before estrogen