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Norepinephrine and temperature regulation in goldfish

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AN ABSTRACT OF THE THESIS OF Lonnie Paul Wollmuth for the
Master of Science in Biology presented November 24, 1987.

Title: Norepinephrine and temperature regulation in
goldfish.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Larry I. Crawshaw, Chairman

Stanley S. Hillman

Randy D. Zelick

Cannulae were implanted into forebrain loci of goldfish
(Carassius auratus; 45-90 g) to determine (i) the effects
and site of action of intracranial norepinephrine (NE)
injections on behavioral thermoregulation and (ii) the
mechanism and the types of adrenoreceptors involved in the
thermoregulatory effect of NE. After 30 min in a thermal
gradient, implanted fish were injected with norepinephrine-
bitartrate salt (2.5-500 ng NE) in a total volume of 0.2 ul
(carrier was 0.7% NaCl). Injections of 5, 10, 25, and 50 ng

NE into the anterior aspect of the nucleus preopticus periventricularis (NPP; Peter and Gill 1975) led to consistent, dose-dependent decreases in selected temperature. No effect on temperature selection was observed following injections of 2.5 ng NE or control injections of 100 ng tartaric acid. The effects of injections into other loci, including intraventricular injections, were dependent upon the dose and proximity to the anterior NPP; at sites adjacent to the anterior NPP, larger doses were required, and the effects became inconsistent. At sites further removed, no effect on selected temperature was observed; included in this category were more caudal sites within the NPP and the nucleus preopticus.

To determine the characteristics of the adrenoreceptors involved in the decrease in selected temperature following microinjections of NE into the anterior NPP, noradrenergic antagonists were injected 10 min prior to an injection of 50 ng NE. In comparison to control injections, injections of 50 ng phentolamine, an alpha antagonist, significantly attenuated the effect of NE. In contrast, 50 ng propranolol, a beta antagonist, produced a non-significant attenuation. These antagonists injected by themselves had no thermoregulatory effect. Comparable thermoregulatory effects were obtained by the following doses of noradrenergic agonists: NE (10-25 ng), clonidine (alpha2,

1.0 ug), phenylephrine (alphal, 5 ug), and isoproterenol (beta, 25 ug).

In goldfish, microinjections of NE into the anterior NPP leads to consistent, dose-dependent decreases in selected temperature. This thermoregulatory effect is limited to the anterior NPP and occurs at doses in the low nanogram range. The alpha-adrenoreceptors appear to be the primary receptor class that infuences thermoregulatory behavior. Since antagonists injected by themselves do not have a thermoregulatory effect, NE may not have a role in the short term regulation of body temperature in fish but rather may play a role in modulating long term responsiveness or adjusting to extreme thermal conditions.

NOREPINEPHRINE AND TEMPERATURE REGULATION

IN GOLDFISH

by

LONNIE PAUL WOLLMUTH

A thesis submitted in partial fulfillment of the
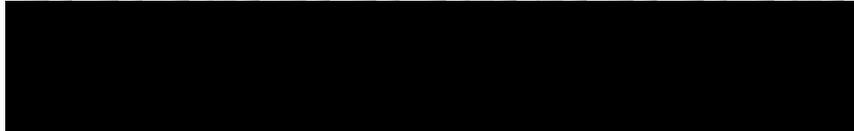requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University

1987

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of
Lonnie Paul Wollmuth presented November 24, 1987.

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Finally, this work is dedicated to Roland and Lenora Wollmuth. Unknowingly, they have been my guide and inspiration throughout my life. This work is a small token in return for everything they have given me.

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CHAPTER I

LITERATURE REVIEW

Temperature has significant effects on physiological processes. Every animal has a temperature range within which its physiological processes function at maximum efficiency (Brett 1971; Dawson 1975). These optimal temperature ranges may vary on a daily or seasonal basis. Temperature extremes, with a wider range than these optimal thermal conditions, can adversely affect the integrity of an animal. To maintain optimal physiological conditions and organismic integrity, animals have the capacity to sense and respond to internal and external thermal fluctuations (Hammel 1968; Crawshaw et al. 1986). These responses are mediated primarily by the central nervous system (Crawshaw 1980).

Based on whether or not metabolic heat production is a significant factor in the maintenance of body temperature, vertebrates are classified as either endothermic or ectothermic. Endothermic vertebrates include birds, mammals, lamnid sharks and tunas. These animals are capable of generating and maintaining high body temperatures through high metabolic rates and the requisite insulative covers. In response to thermal challenges, body temperature is

maintained both autonomically (e.g., vascular tone, metabolic rate, and changes in evaporative water loss) and behaviorally (e.g., moving to a favorable microhabitat). Ectothermic vertebrates include reptiles, amphibians and all fishes except those mentioned above. Because of low metabolic rates and poor insulative covers, behavior is the primary means body temperature is regulated in these animals. In amphibians and reptiles, behavioral thermoregulation can include the selection of an appropriate ambient temperature as well as postural adjustments to optimize thermal inputs from the substrate and/or the sun. In contrast, ectothermic fishes regulate body temperature solely through the selection of an appropriate thermal environment. Because of the high heat capacity of water, the body temperatures of ectothermic fishes, in general, is very close to ambient thermal conditions.

In the ectothermic fishes, as in the endothermic vertebrates, a primary site of the neural circuitry involved in behavioral thermoregulation has been grossly localized, via thermode and lesion studies, to the anterior brainstem (Hammel 1968; Nelson and Prosser 1979; Crawshaw 1980). In thermode studies, local brain temperatures are manipulated by stradling thermodes, hollow tubes connected to temperature baths, across the brain. The thermal sensitivity of an area is reflected by how a unit change in brain temperature affects thermoregulatory behavior. In

lesion studies, particular brain regions are ablated, and the subsequent effect on thermoregulatory behavior ascertained.

The thermal sensitivity of the anterior brainstem has been demonstrated in a number of ectothermic fishes, including, for example, arctic (Hammel et al. 1969), antarctic (Crawshaw and Hammel 1971) and temperate (Crawshaw and Hammel 1974) teleosts, and sharks (Crawshaw and Hammel 1973). For example, in the Brown bullhead (Ictalurus nebulosus), if the anterior brainstem temperature was increased by progressive amounts, a proportional decrease in the selected temperature (and hence body temperature) occurred (Crawshaw and Hammel 1974). On the other hand, warming more caudal sites either had no thermoregulatory effect or required much higher temperature changes to obtain similar responses.

Although thermode studies demonstrate the thermal sensitivity of the anterior brainstem in ectothermic fishes, they make only a gross localization of the sites involved in this response since a number of prominent nuclei are interspersed between the thermodes, including the hypothalamus and the septum. Both of these anatomical areas have been shown to participate in the control of body temperature in endothermic vertebrates (Boulant 1980), but such conclusions were arrived at with techniques other than thermodes.

More restricted localizations of central sites involved in thermoregulation have been accomplished by lesion studies. Following lesions of the medial and lateral preoptic areas, the thermoregulatory behavior of the green sunfish (Lepomis cyanellus) and the goldfish (Carassius auratus), in comparison to sham operated controls, was either severely or totally disrupted (Nelson and Prosser 1979). However, although the lesions in this study are more restricted than the areas affected by thermodes, large areas of the brain are still ablated, and discrete localization is thus precluded.

In endothermic vertebrates, single unit recordings of temperature sensitive cells and chemical stimulation have also been utilized to characterize the nervous control of body temperature. Similar studies in aquatic ectotherms are quite limited.

Nelson and Prosser (1981) demonstrated the presence of several classes of warm-sensitive cells and one class of cold-sensitive cells within the preoptic area of the sunfish. These cells appear to be located in the nucleus preopticus (NPO; Peter and Gill 1975) and had sensitivities similar to those observed in endothermic vertebrates. Warm- and cold-sensitive cells have also been found in the brainstem of brook trout (Greer and Gardner 1970).

In endothermic vertebrates, norepinephrine (NE), serotonin (5-HT), dopamine (DA) and acetylcholine (ACh) have

been suggested to play a crucial role in the regulation of body temperature (Myers 1980). In ectothermic fishes, few studies have examined the neurochemistry of thermoregulation. The fish *Cromus chromus*, after intracerebral injections of cholinergic substances, both nicotinic and muscarinic, tolerated lower temperatures before escaping into surrounding, higher temperature water than did controls (Green and Lomax 1976). Immersion of the same species in L-Dopa resulted in the opposite response. These two findings suggest that cholinergic systems lower the thermoregulatory set-point, while catecholaminergic systems increase the thermoregulatory set-point.

In conclusion, despite information on the gross localization of thermoregulatory centers, little is known about the precise localization or the neurochemistry of thermoregulation in ecothermic fishes. The few studies involving neurotransmitters have not localized discrete areas participating in thermoregulatory responses.

CHAPTER II

INTRODUCTION

In endothermic vertebrates, norepinephrine (NE) was one of the initial neurotransmitters to be implicated in the regulation of body temperature (Feldberg and Myers 1965). While the role of NE remains somewhat controversial (Satinoff 1979), many studies indicate that it activates, either directly or indirectly, the effector systems involved in heat dissipation (Gisolfi and Christman 1980; Christman and Gisolfi 1985). In contrast, a role for NE in the control of thermoregulation in ectothermic fishes remains undefined. Immersion of fish (<u>Chromus chromus</u>) in water containing L-Dopa leads to an increase in escape temperatures (Green and Lomax 1976). Although the dopaminergic or noradrenergic component of this response was not distinguished, the results suggest that catecholamines may have a role in increasing the thermoregulatory set-point in fish.

Since immersion studies result in a broad distribution of the neurotransmitter, many physiological systems can be affected. Hence, thermoregulatory behavior can be disturbed in various ways, and precise localization of the effect is impossible. For these reasons, the following study on NE

and temperature regulation in fish was pursued. This study
is divided into two parts. In the first part, we injected
small volumes of NE into discrete brain loci in goldfish
(_Carassius_ _auratus_) and evaluated the effect on temperature
regulation. In the second part, the characteristics of the
adrenoreceptors involved in the thermoregulatory response
were determined.

CHAPTER III

MATERIALS AND METHODS

ANIMALS

Goldfish (_Carassius auratus_) of 45-90 g were
maintained at 25 C with a 12L:12D photoperiod for a minimum
of seven days before, and throughout the period of,
experimentation. The fish were fed each afternoon, and
water in the tanks was changed as needed. All water used in
the maintenance tanks and the temperature gradient was
dechlorinated, and since Portland municipal water is very
soft, the electrolyte levels were supplemented with 2.5 mg
NaCl and 0.26 mg KI per liter.

TEMPERATURE SELECTION MEASUREMENTS

Temperature selection was monitored in an aquatic
thermal gradient device. This device consisted of nine
adjacent, separate temperature gradient lanes, each of about
240 cm X 24 cm X 16 cm deep. Submerged in both ends of each
lane were heat exchangers coupled by circulating pumps to
either a hot or a cold source. Each lane was partially
divided into ten equal chambers by baffles, and each chamber
was vigorously aerated. Two hours after the gradient was
turned on, nine nearly linear thermal gradients of about 7 C

to 35 C were established. Continuous monitoring showed that any point in the gradient was stable to within ±0.5 C for the duration of the experiment. The temperature profile for each gradient was determined by measuring the temperature at the geometric center of each chamber at the end of each day.

Positions of fish in the nine lanes were imaged using a wide angle camera located above the gradients and digitized by an Occulus frame grabber. This information was processed, at 5 second intervals, by an IBM PC XT which stored tha data and provided an output to plot the position of each animal, thus generating a real-time graph of their position. After the experiment, these stored position values were transformed into temperature values by using the calibration data for the appropriate lane. The temperature difference between successive data points was used as a measure of the movement of the fish in the gradient (1 activity unit = change in selected temperature in degrees C between successive 5 second intervals). This method of measuring activity has been used previously (Crawshaw 1975).

SURGICAL PROCEDURE

Twenty-four hours before surgery, fish were habituated to the gradient for 5 hours. On the day of surgery, fish were weighed, anesthetized in MS-222 (3500:1, w/w; pH adjusted to 7.0), and then placed in a Baltimore instruments stereotaxic device adapted for fish (Peter and Gill 1975).

The gills of the fish were perfused throughout the surgery with an MS-222 solution (10,000:1; pH adjusted to 7.0). The cranium and fatty substances directly superior to the forebrain were then removed, and a guide cannula (26 ga.) and indwelling stylette implanted. Landmarks on the surface of the brain were used as references for placing the cannula. The cannula was secured, with dental acrylic, to vitallium bone screws placed in the skull. This system allowed repeated injections into specific brain loci. All cannulae, stylettes, and screws were sterilized with 70% ethyl alcohol and then rinsed with distilled water prior to use.

Following surgery, the goldfish were able to swim and feed normally within 15 minutes. Twenty-four hours after surgery the fish were placed in the gradient for 4 hours to verify that thermoregulatory behavior was similar to pre-surgery patterns. Injections were started 48 hours after surgeries.

INTRACRANIAL INJECTIONS

On the day of an experiment, goldfish were placed in the thermal gradient where the temperature was 25 C and allowed to select temperatures for approximately 20 minutes. The next 30 minutes was recorded for use as baseline data, after which the fish were captured. The indwelling stylette was then removed and replaced with a 33 ga. injection

cannula, attached by P.E. 20 tubing to a 5 μl syringe. The
fish were then placed in an enclosure located at 25 C for 10
minutes, during which injections were made (see below). At
the end of this 10 minute interval, the P.E. tubing was
simultaneously sealed and cut with hot forceps, the
enclosures quickly removed, and the fish allowed to swim
free. Position of the goldfish was subsequently recorded
for 30 minutes. Individual fish were injected every other
day.

Depending on the substances injected, one of two
injection procedures were utilized during the 10 minute
period the fish were in the enclosure. When the agonists or
the antagonists by themselves were injected, the substance
was injected at the end of the 10 minute period. When
attempting to block the noradrenergic decrease in selected
temperature, the antagonist was injected at the start of the
10 minute period and 50 ng NE at the end of the 10 minute
period. Fifty ng NE was used as a test dose since, although
the decrease in selected temperature is robust, the effect
is over within 30 minutes (see Results section, Effect and
Site of Action).

Norepinephrine-bitartrate salt, clonidine HCl,
phenylephrine HCl, and isoproterenol HCl were obtained from
Sigma Co. Phentolamine HCl was obtained from CIBA-Geigy.
Injection doses are given as the agonist or antagonist
exclusive of the complexing agent and were made fresh daily.

The vehicle, 0.7% NaCl, was passed through a 0.22 μm
millipore filter system prior to dilution.   Control
injections for NE utilized 100 ng tartaric acid; this
solution had the same pH (3.1) as a 500 ng NE solution.
Agonist and antagonist control solutions used 0.7% NaCl,
with the pH adjusted to 3.0 utilizing 0.1 N HCl; this pH
corresponds to the pH of the most acidic injection solution.
All injections had a total volume of 0.2 μl and were
dispensed over several minutes.

HISTOLOGY

After a maximum of five intracranial injections, the
fish were anaesthetized and the circulatory system perfused
with 5 ml 0.1 M phosphate buffer (pH=7.4) and then with 5 ml
2.5% gluteraldehyde in the same phosphate buffer solution.
After the perfusion, 0.2 ul of Evans blue dye was injected
through the injection cannula.   The brain was then removed
and placed in fixative for 2-3 hours at 4 C, washed for 10
minutes in phosphate buffer, and placed in 30% sucrose (in
0.1 M phosphate buffer) for at least 24 hours.   After a 10
minute wash in phosphate buffer, the brain was embedded in
O.C.T. compound, frozen with liquid CO2, and sliced in the
transverse plane at 6 um on a cryostat.   Slices were taken
over the area stained by the dye and were placed on gelatin
subbed slides, stained with methylene blue-azure A,
counterstained with 0.05% basic fuchsin, and covered.

The location of the injection site was determined by the tract left by the guide and injection cannulas. The site of injection was taken to be the center of the tip of the injection cannula tract. Ventricular injections were indicated by the presence of dye in the ventricle, and the rostro-caudal location determined by tissue damage from the guide cannula.

DATA ANALYSIS

To evaluate the effect of an injection, we established the Temperature Selection Index (TSI). This index incorporates both the magnitude and duration of post-injection changes in selected temperature into a single numerical value. To determine the TSI, the mean 30 minute pre-injection selected temperature was subtracted from each post-injection temperature (taken at 5 second intervals). Then, starting with the first post-injection observation, these differences were summed until the animal's selected temperature was within 2.5 C of the pre-injection value for 4 consecutive minutes; differences occurring during this final 4 minute window were not included in the summed value. For the sake of convenience, this summed value was divided by 1000. As an example, if for a 10 minute period following an injection the temperature selected was 10 C below the pre-injection mean and then abruptly returned to pre-injection levels, the summed value would be -1200 (based

on 120 total observations), and the TSI would be -1.2. For certain injection sites (i.e., those nearby, but not in the most sensitive area), a delayed thermoregulatory response was observed. In these cases, the thermoregulatory response was determined by summing the differences which were bordered on either side by the 4 minute ±2.5 C window, and the time after injection when this change occurred was noted.

To quantify the effects of NE on activity, the activity units during the TSI interval were summed, and this sum was divided by the number of observations included in the TSI interval to obtain the mean level of activity. When there was no effect on selected temperature or when the effect lasted less than 6 minute, the mean activity during the first 15 minute post-injection was used.

The information obtained for each fish was related to the intracranial injection site. An ANOVA was used to test for statistical differences in both activity and TSI for NE injections into highly effective sites. A Student's t-test was used to test for statistical differences of the thermoregulatory effect of 50 ng NE either injected by itself or following the control solution. An ANOVA was used to test for statistical differences in the effects of the antagonists injected before NE, the antagonists injected by themselves, and the agonists. When necessary, the Tukey

test was used for multiple comparisons. Unless otherwise noted, significance was assumed if P < 0.05.

CHAPTER IV

RESULTS

EFFECTS AND SITES OF ACTIONS

A total of 120 NE and control injections were made on
53 different fish. The mean pre-injection selected
temperature was 24.7±0.2 C (N=120). During this interval,
activity showed a mean value of 1.19±0.06 activity units.

The effect of NE injections on temperature selection
was dependent upon the dose and neuroanatomical location of
the injection site. The injection site was identified in
all fish. Four sites were within the third ventricle and
the remaining 49 were in the forebrain. The area most
sensitive to NE injections was the anterior aspect of the
nucleus preopticus periventricularis (NPP; Peter and Gill
1975). Typical results for NE and for control injections
into this area are shown in Figure 1, which contains
records, at 2 minutes intervals, of pre- and post-injection
selected temperature for fish injected with 50 ng NE, 10 ng
NE, and 100 ng tartaric acid. Injections of NE into the
anterior NPP produced a consistent decrease in selected
temperature, as demonstrated by the top two records. In
contrast, control injections, as shown in the bottom record,
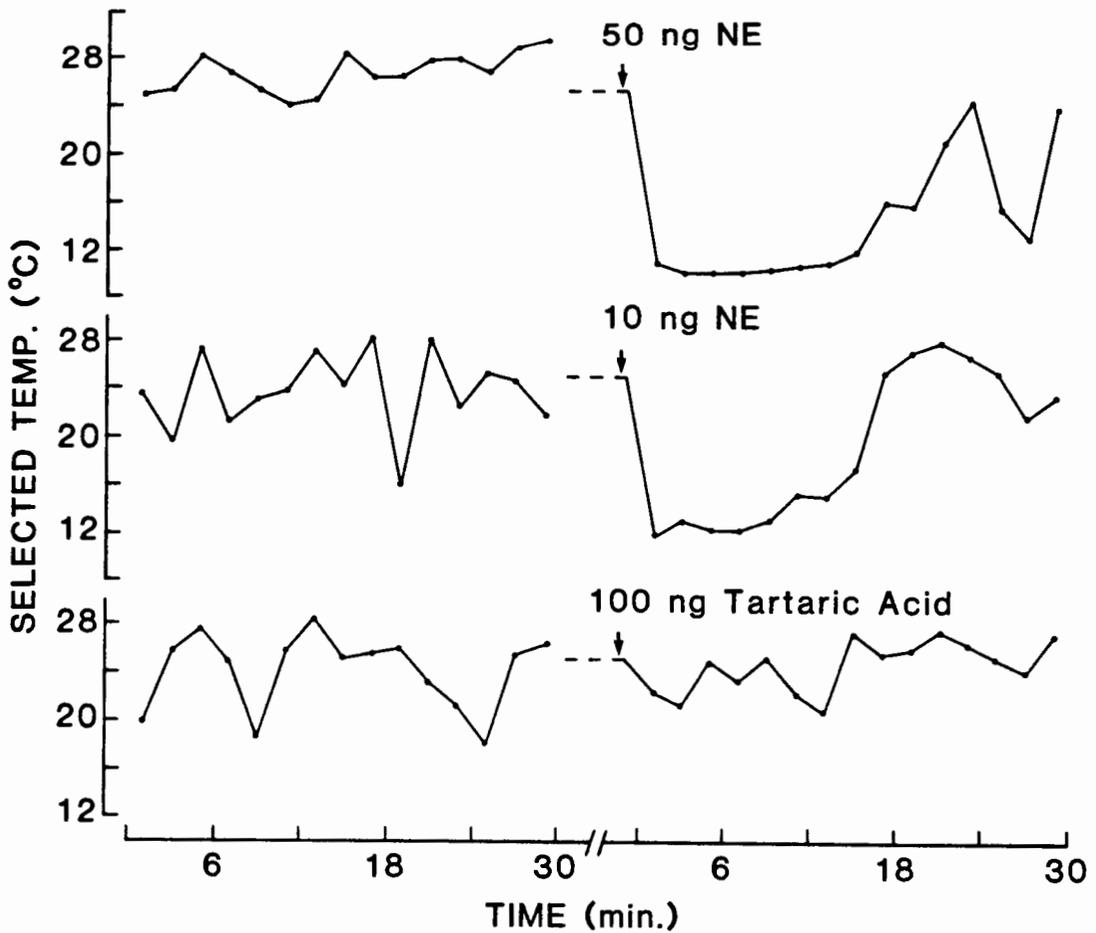had no effect on temperature selection.

Figure 1. Pre- and post-injection selected temperature (at 2 min intervals) for injections into the anterior aspect of the nucleus preopticus periventricularis (NPP). The dashed lines indicate the 10 min period when the animal was caught, the injector inserted, and the fish placed at 25 C and allowed to settle down. The arrows indicate the time of injection.

All twelve fish implanted in the anterior NPP were
responsive to NE injections. A total of 30 NE and control
injections were made in the anterior NPP; a dose-response
curve for these injections is shown in Figure 2. Data from
control injections are also included in this figure for
comparison. At 2.5 ng NE, three fish showed no change in
selected temperature, one fish showed a small decrease in
selected temperature, and one showed an increase in selected
temperature. This latter fish, however, had a low mean
pre-injection selected temperature (21.9 C), and the mean
selected temperature during the post-injection interval was
not extreme (26.5 C). Three of four fish injected with 5 ng
NE showed decreases in selected temperature, while the
fourth fish showed no change. At doses of 10, 25 and 50 ng
NE, an immediate decrease in selected temperature was
observed, without exception, for fish injected in the
anterior NPP. The slope of the regression line for this
dose-response curve was significantly different from 0 ($P <$
0.001, df = 24). Doses above 50 ng NE are not included in
this curve. At this high dose, several fish (3 of 7)
selected and remained in very cold water for so long that
they became disoriented and were unable to behaviourally
thermoregulate.

A significant decrease was observed in activity levels
following injections into the anterior NPP ($P < 0.05$, df =
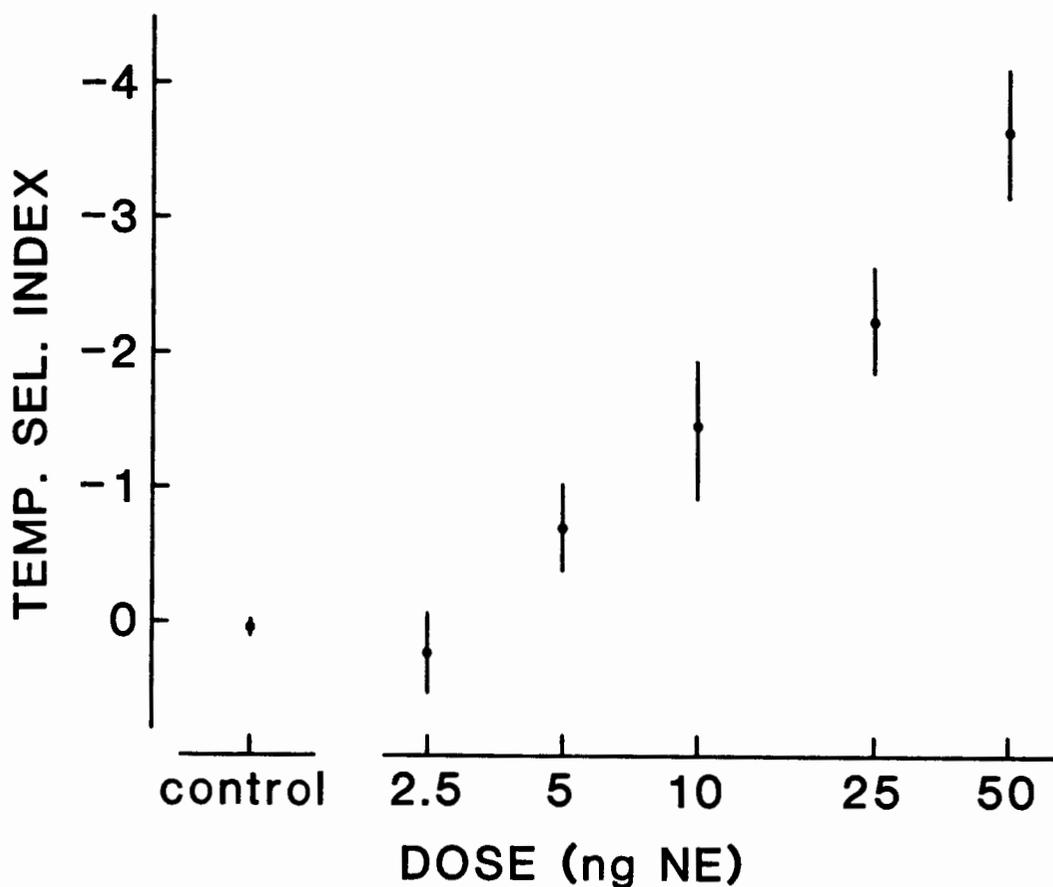24). Multiple comparison between groups showed that no

Figure 2. A semi-logarithmic dose-response curve
for NE injections into the anterior NPP.
Starting with the lowest dose, the number of
injections at each dose was 5, 4, 5, 5, and 7.
Control injection data are included for
comparison (N=4). Values shown are means±SEM.
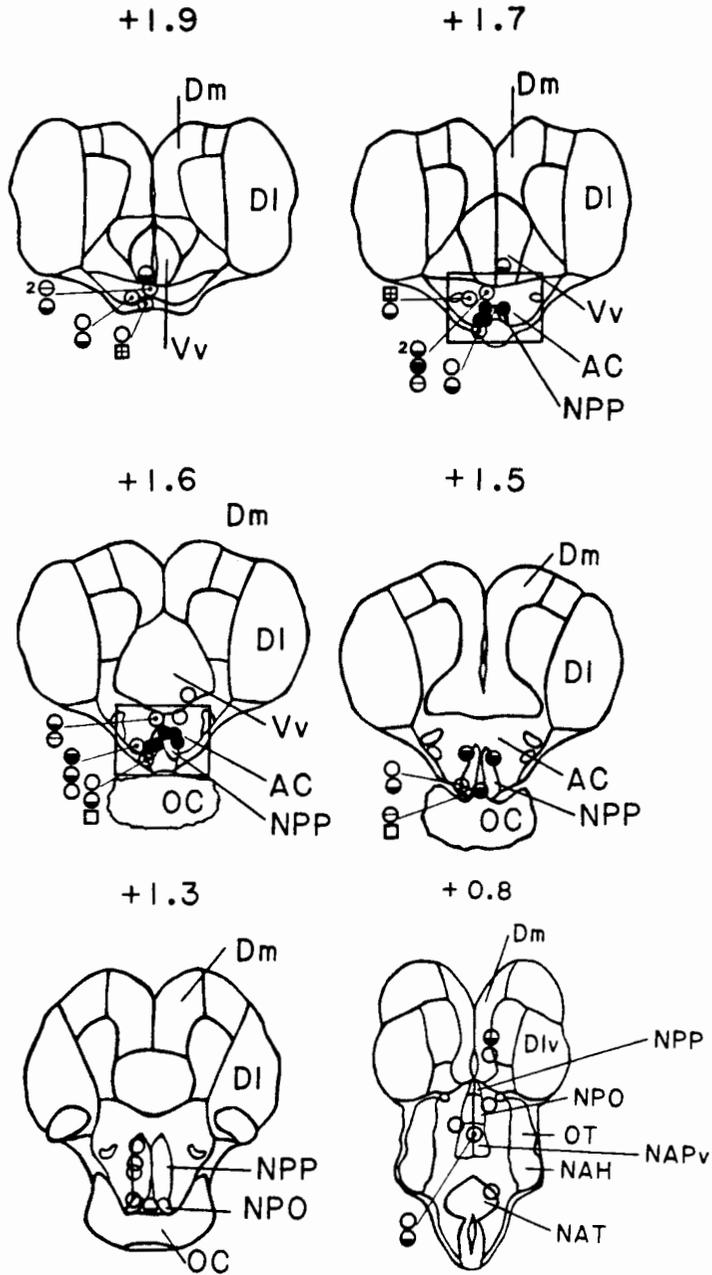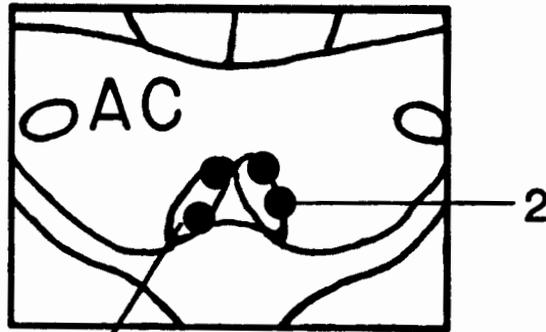Twelve different fish were implanted in the
anterior NPP.

Figure 3. Effects of NE injections at different
sites in the goldfish forebrain. The transverse
plates are taken form Peter and Gill (1975).
Plate +1.9 includes sites from +2.0 to +1.8,
plate +1.3 includes sites from +1.4 to +1.2, and
plate +0.8 includes sites from +1.0 to +0.6.
Twelve implanted fish were very sensitive to NE
injections (solid circles). The injection sites
of these animals were all within the anterior
NPP; there were no ineffective or partially
effective sites within the anterior NPP. The
boxed areas in plates +1.7 and +1.6 contain all
of the sensitive sites, whose exact position is
displayed in Fig. 4. To relate the decreases in
selected temperature observed at other sites to
those in the anterior NPP, the former effects
were expressed as a percentage of the mean effect
observed in the anterior NPP. Effects with
25-50% of the magnitude of the mean effect
observed in the anterior NPP are indicated by a
three-quarters darkened circle. Effects with
less than 25% of the magnitude are indicated by
an one-quarter darkened circle. Both of the
above partial effects were often not
dose-dependant. An open circle denotes no
effect. The selection of higher temperatures
following NE injections is indicated by an open
square. Delayed effects on temperature selection
following injections are indicated by a minus in
a circle (decrease) or a plus in a square
(increase). More than one type of effect at a
particular injection site is indicated by a
concentric circle; the various effects are
displayed outside the transverse section, with
the more predominant effects at the top. Numbers
indicate that more than one animal was injected
at a particular site. Abbreviations-- AC,
anterior commissure; Dl, area dorsalis
telencephali pars lateralis; Dlv, area dorsalis
telencephali pars lateralis ventralis; Dm, area
dorsalis telencephali pars medialis; NAH, nucleus
anterioris hypothalami; NAPv, nucleus anterioris
periventricularis; NAT, nucleus anterior tuberis;
NPO, nucleus preopticus; OC, optic chiasm; OT,
optic tract; Vv, area ventralis telencephali pars
ventralis.

significant difference existed between the controls (x±SE: 1.13±0.34, n=4) and the lower NE doses, 2.5 ng NE (1.06±0.28, n=5) and 5 ng NE (0.8±0.22, n=4); in contrast, the three higher doses, 10 ng NE (0.41±0.09, n=5), 25 ng NE (0.46±0.1, n=5), and 50 ng NE (0.37±0.09, n=7), were significantly different from the controls and lower doses, but were not significantly different from each other (P < 0.05).

The effects of NE injections into all forebrain loci tested are shown in Figure 3 on tranverse plates from Peter and Gill (1975). The boxed areas in plates +1.7 and +1.6, which contain the most sensitive sites to NE injections (solid circles), are enlarged and redisplayed in more detail in Figure 4. As discussed above, these sites were all localized within the anterior NPP.

At injection sites not in the anterior NPP, doses of 10-500 ng NE were tested. Injections into sites rostral to the anterior NPP, as shown in plate +1.9, were generally associated with minor decreases in selected temperature either immediately following the injection or with a delay of 6-10 minutes. These effects, if any, occurred with higher doses (100-500 ng NE), had a magnitude comparable to the injection of 5 ng NE into the anterior NPP, and were not necessarily dose-dependent. For plates +1.7 and +1.6, injections into sites not in the NPP showed a similar pattern. However, at sites progressively closer to the NPP,
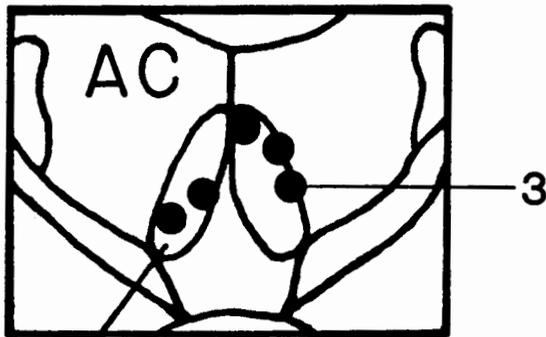
Figure 4. Neuroanatomical location location of NE injections into the anterior NPP. These enlarged sections are the boxed areas in Fig 3. Numbers indicates that more than one fish was implanted at this site. Abbreviations as in Fig. 3.

effects were observed at lower doses (50-100 ng NE), and the magnitude of the response was comparable to anterior NPP injections of 10 ng NE.

The effect of NE injections into the NPP decreases rapidly as one moves caudally from plate +1.6. In plate +1.5, injections of 100 ng NE into the dorsal aspect of the NPP lead to decreased temperature selection comparable to a dose of 25 or 50 ng NE injected into the anterior NPP. On the other hand, injections of 10 ng NE into these caudal sites were without a thermoregulatory effect. Injections into more ventral aspects of the NPP produced irregular effects that were not dose-dependant. At sites caudal to plate +1.5, injections into the NPP or the nucleus preopticus (NPO), as shown in plates +1.3 and +0.8, were not associated with any change in selected temperature.

Intraventricular injections of NE, as shown in plates +1.5, +1.3, and +0.8, were associated with decreases in selected temperature. At the most rostral site, a decrease in selected temperature was observed at doses of 50 ng and above, but the magnitude of the effect was not dose-dependent. More caudally, a decrease in selected temperature, if any, was observed only at 500 ng NE.

ADRENORECEPTORS

A total of 120 fish were implanted. Of these, the
injection site was localized to the anterior NPP in 27 fish.
All of these fish showed a decrease in selected temperature
following an injection of NE. A total of 101 injections
were made on these fish. The mean pre-injection selected
temperature for these animals was 24.9±0.2 C.

Figure 5 shows records of pre- and post-injection
selected temperature, at 2 minute intervals, for fish
injected into the anterior NPP with 50 ng phentolamine, 50
ng propranolol, and the control solution 10 minutes prior to
the injection of 50 ng NE. In comparison to the control
injection, 50 ng phentolamine, an alpha-adrenergic
antagonist, greatly attenuated the decrease in selected
temperature associated with 50 ng NE. The injection of 50
ng propranolol, a beta-adrenergic antagonist, partially
attenuated the effect of 50 ng NE.

The left half of Figure 6 shows group data for the
injection of the noradrenergic antagonists or the control
solution 10 minutes prior to the injection of 50 ng NE.
Injecting the control solution 10 minutes prior to 50 ng NE
(-3.18±1.2, n=7; x±SEM) resulted in thermoregulatory effects
not significantly different from the injection of 50 ng NE
by itself (-2.43±0.25, n=20). In contrast, the injection of
50 ng phentolamine prior to the injection of 50 ng NE, in
all instances, either greatly attenuated or completely
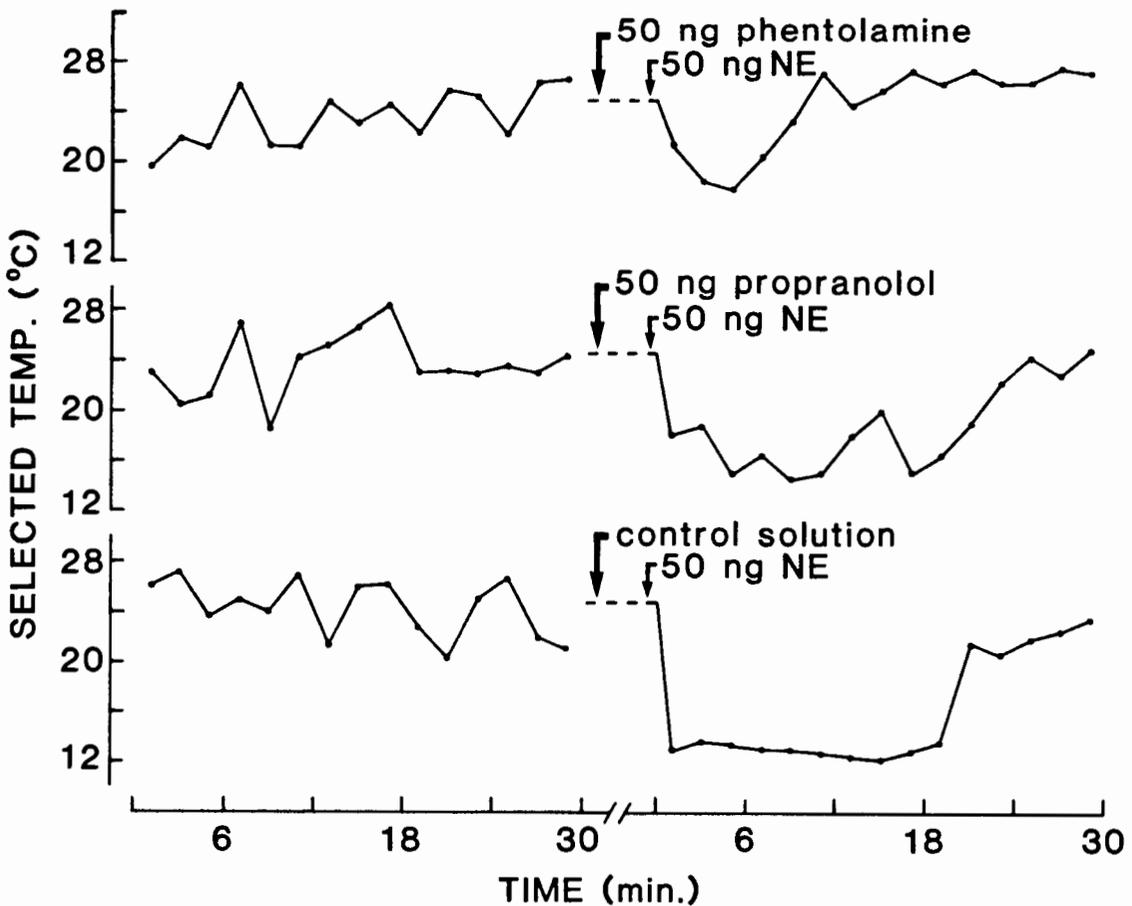
Figure 5. Pre- and post-injection selected
temperature (at 2 min intervals) for injections
of noradrenergic antagonists or the control
solution prior to 50 ng NE into the anterior NPP.
The dashed lines indicate the period when the
animal was caught, the injector inserted, and the
fish placed at 25 C. Phentolamine, an alpha
antagonist, propranolol, a beta antagonist, and
the control solution (bold arrows) were
pre-injected 10 min prior to the injection of 50
ng NE (narrow arrows).

Figure 6. Group data for fish injected with control solution, 50 ng phentolamine, or 50 ng propranolol either 10 min before the injection of 50 ng NE (left half of figure) or by themselves (right side of figure). Values shown are means+SEM. From left to right, the number of animals injected was 7, 7, 6, 6, 4 and 4.

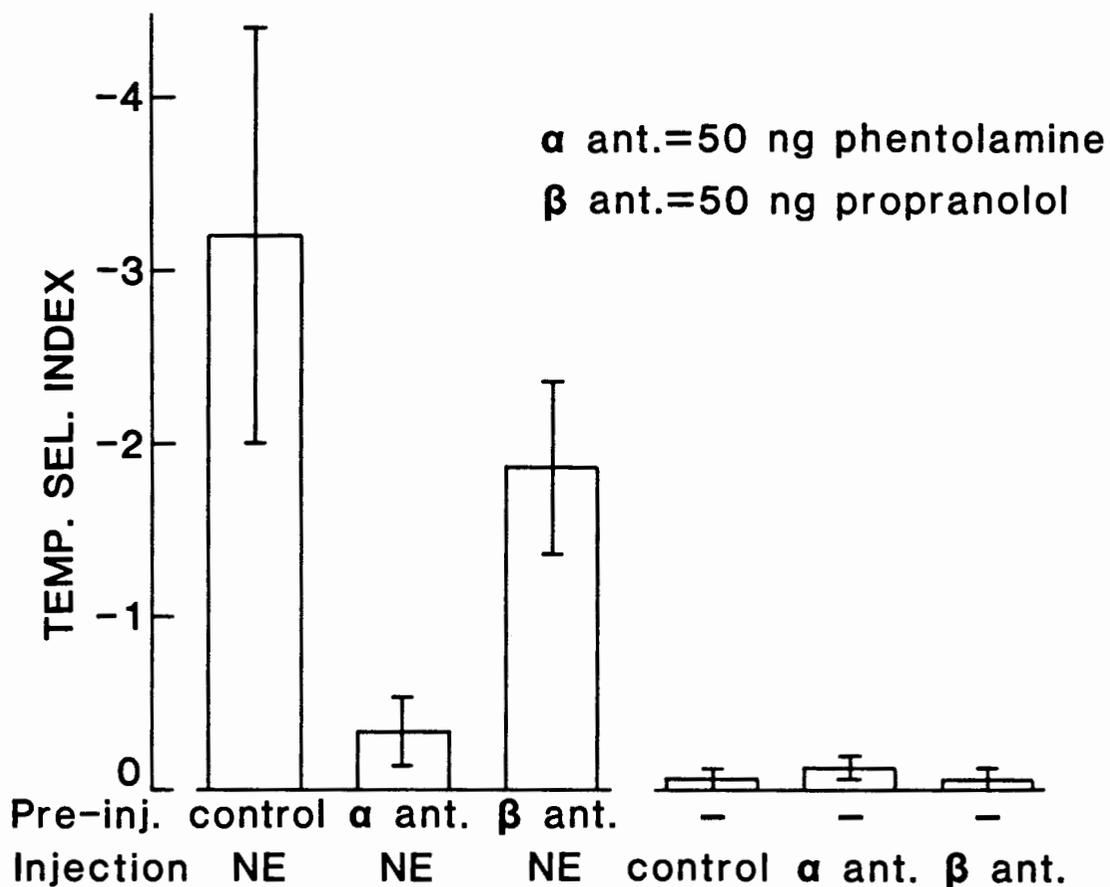abolished the decrease in selected temeperature associated with 50 ng NE (−0.31±0.2, n=7). The effect of 50 ng NE was partially attenuated by 50 ng propranolol (−1.88±0.5, n=6). The thermoregulatory effects of 50 ng NE following the injection of the antagonists or control solution were significantly different (P < 0.001, df=17). Multiple comparisons between groups showed that the attenuated response with 50 ng phentolamine was significantly different from both the response of control injections (q=7.0, P < 0.001) and 50 ng propranolol (q=3.67, P < 0.05). The response with 50 ng propranolol was not significantly different from the controls.

The right half of Figure 6 shows the thermoregulatory effect of the noradrenergic antagonists or control solutions injected by themselves at the end of the 10 minute restraint interval. No significant differences were observed between any of the groups, nor were any of the responses significantly different from zero.

Figure 7 shows dose-response curves for the injection of various noradrenergic agonists into the anterior NPP. Data from control injections are also included in this figure for comparison. The dose-response curve for NE is taken from Figure 2. Of the various adrenoreceptor agonists, clonidine, an alpha2 agonist, was the most efficacious, followed by phenylephrine, an alpha1 agonist, and isoproterenol, a beta agonist. The slopes for clonidine
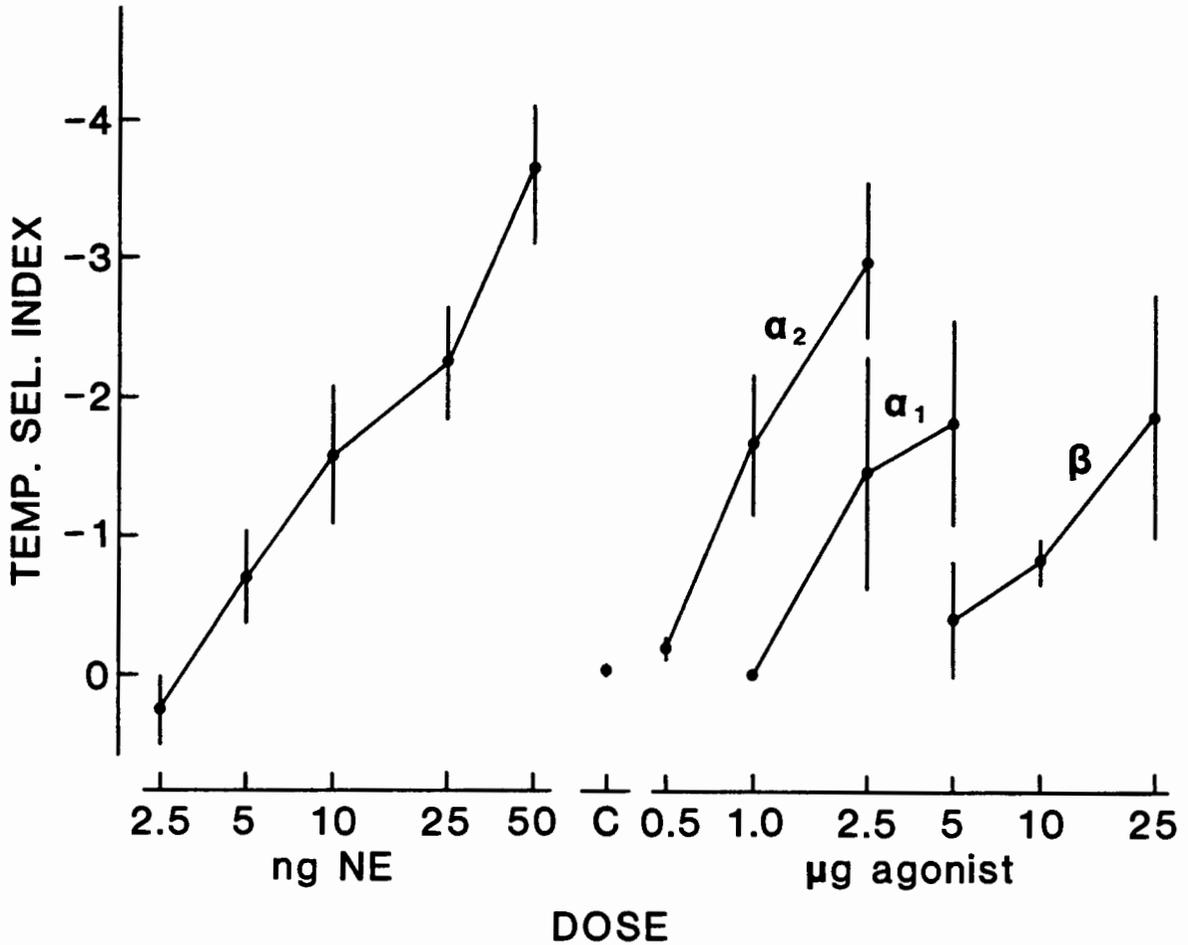
Figure 7. Semi-logarithmic dose-response curves for noradrenergic agonists injected into the anterior NPP. The NE dose-response curve is from Figure 2. Starting with the lowest dose, the number of injections at each dose for the agonists are clonidine (5, 6, 4), phenylephrine (4, 4, 5) and isoproterenol (6, 4, 5). Control injection data are included for comparison (N=6). Values shown are means±SE.

(P < 0.001, df=13) and phenylephrine (P < 0.02, df=15) were significantly different from 0; the slope for isoproterenol was not significantly different from 0 (P < 0.10, df=13).

CHAPTER V

DISCUSSION

EFFECTS AND SITE OF ACTION

The mean pre-injection selected temperature was
comparable to the acclimation temperature and similar to
temperatures selected by adult goldfish left in a gradient
for a long period of time (Coutant 1977). Injections of
5-50 ng NE into the anterior NPP led to a consistent,
dose-dependent decrease in selected temperature. The
effects of injections into other brain loci were dependent
upon the dose and proximity to the anterior NPP. Other work
has demonstrated the presence of catecholaminergic terminals
in this portion of the forebrain. Using the Falck-Hillarp
histofluorescence technique, Parent et al. (1978) observed
catecholaminergic collaterals of fibers projecting to the
telencephalon which branched within the neuropil of the NPP
in the pumpkinseed sunfish (Lepomis gibbosus). The
dopaminergic and noradrenergic fibers were not distinguished
in this study. The authors suggest, however, that since
this region contains a higher proportion of NE than
dopamine, most of these fibers are noradrenergic and may
arise from a structure homologous to the locus coeruleus in
reptiles and endothermic vertebrates. In mammals, the

hypothalamic projection from the coeruleus region, in particular the subcoeruleus, provides a noradrenergic input to thermoregulatory centers (Bruck and Hinckel 1982).

The location of thermoregulatory centers in fish was initially demonstrated by thermode studies which showed that changes in the local temperature of the anterior brainstem of fish led to compensatory changes in behavior (Crawshaw and Hammel 1974; Hammel et al. 1969). Subsequently, Nelson and Prosser (1979) observed that bilateral lesions in the medial preoptic region of goldfish or green sunfish (Lepomis cyanellus) led to random behavior in a thermal gradient, while sham operated controls selected temperatures in a normal manner. Lesion sites which led to random behavior, while too large to localize to discrete nuclei, appear to include the NPP and the rostral NPO. In addition, electrophysiological recordings by Nelson and Prosser (1981) demonstrated the presence of warm- and cold-sensitive neurons within the preoptic region of the sunfish, most of which were reported to be in the NPO. Although we injected high doses of NE throughout the NPP and the NPO, only within the anterior NPP was a consistent thermoregulatory effect observed. A similar rostro-caudal distinction has been observed for the thermoregulatory effects of NE in mammals (Metcalf and Myers 1978).

Most studies on the role of NE in thermoregulation have been carried out in endothermic vertebrates. In birds,

intrahypothalamic injections of NE at low ambient
temperatures (6 C) caused a decrease in shivering and hence
a fall in body temperature (Hissa and Rautenberg 1974); in
contrast, similar injections at higher ambient temperatures
(38 C and 42 C) had no effect on body temperature (Pyornila
et al. 1978). For most mammalian species, injections of NE
into the preoptic/anterior hypothalamus (POAH) lead to a
decrease in body temperature (Bruinvels 1979; Gisolfi and
Christman 1980). Initially, it was proposed that normal
body temperature was maintained by the balanced release of
NE and serotonin (5-HT) within the POAH (Feldberg and Myers
1965). However, this model has been questioned since
depletion of hypothalamic monamines does not affect the
regulation of normal body temperature (Feldberg 1975).
Nevertheless, considerable evidence has accumulated to
suggest that NE participates in the central mechanism,
either directly or indirectly, of heat dissipation. Thus,
the release of labeled NE from the POAH region is increased
when the animal is warmed, but unaffected when the animal is
cooled (Myers and Chinn 1973). When the catecholamine-
containing neurons in the POAH are lesioned with
6-hydroxydopamine (6-OHDA), thermoregulation in the heat is
impaired, and body temperature rises (Myers and Ruwe 1977).
In addition, injections of phentolamine, an alpha-adrenergic
blocker, into the anterior hypothalamus leads to increased
hyperthermia during excercise (Gisolfi and Christman 1980).

Also, levels of stored NE in the preoptic area are higher in heat acclimated than in control animals (Christman and Gisolfi 1985).

In the rat, hypothermia, hyperthermia and biphasic responses have been observed following intracerebral NE injections (Beckman 1970; Veale and Whitsow 1976). Satinoff (1979) suggests that the hypothermia observed following NE injections may be the result of activation of heat-loss effector systems and not the result of changes in the thermoregulatory set-point. This conclusion is based on experiments where the animal had a decrease in body temperature following NE injections but compensated behaviorally by increasing ambient temperatures. Further, Satinoff (1979) suggests that NE may participate in increasing the set-point since in several experiments where increased body temperature was observed, a coordinated physiological and behavioral response occurred. Gisolfi and Christman (1980) also address the disparity in the effects of intracranial injections of NE in the rat. These authors suggest that anatomical differences may account for most of the variation observed, with injections into the preoptic region leading to hyperthermia and injections into the anterior hypothalamus resulting in hypothermia. In our experiments, occasional increases in temperature selection were observed following NE injections. However, this response occurred very infrequently, was not dose-dependent,

and was typically observed following a low pre-injection selected temperature.

In the rat, the medial preoptic area is important in the regulation of body temperature, sexual behavior, and endocrine functions. In goldfish, similar functions have been associated with the anterior NPP. Estrogen concentrating cells (Kim et al. 1978) and luteinizing hormone-releasing hormone containing cell bodies (Kah et al. 1983) have been localized to the anterior NPP. Further, lesions in the anterior NPP leads to increases in serum gonadotropin levels (Peter and Paulencu 1980) and decreases in spawning and courtship behavior (Kyle and Peters 1982). Previous thermode and lesion studies implicate this vicinity, and the present microinjection study specifically demonstrates the importance of the anterior NPP in temperature regulation in fish. Since reproductive events in goldfish are dependent on temperature shifts (Stacey 1983), the anterior NPP may be an important site of interaction between thermoregulatory and reproductive systems.

Following higher doses of NE within the anterior NPP, activity levels declined significantly. This change in behavior could result if the anterior NPP is important in the regulation of the overall activity level. Alternatively, the effect could be the result of a large divergence between body temperature (25 C) and the regulated

temperature following the NE injection (approximately 15 C).
During this period of thermal equilibration,
thermoregulatory behavior, because of the large error
signal, may have temporarily suppressed other behaviors such
as exploration or food seeking.

ADRENORECEPTORS

Microinjections of NE either by itself or after the
injection of the control solution resulted in a rapid and
reversible decrease in selected temperature. This effect
occurred only following injections in the anterior NPP. The
alpha-receptor blocker phentolamine, injected prior to NE,
abolished or greatly attenuated this response; in contrast,
the beta-antagonist propranolol produced a non-significant
attenuation. Injections of noradrenergic agonists
corroborated the predominance of alpha-adrenoreceptors in
eliciting the decrease in selected temperature. Of these,
the alpha2 agonist, clonidine, was the most effective.

In the central nervous system of mammals, alpha2-
adrenoreceptors are associated with post-synaptic inhibition
or, as autoreceptors, pre-synaptic inhibition (Rogawski
1985). However, the mechanism of activity of the
adrenoreceptors in fish and other ectothermic vertebrates
has not been described in detail. Further, alpha1-
adrenoreceptors are associated with post-synaptic excitation
(Rogawski 1985), yet activation of these receptors produces

thermoregulatory effects in the same direction as activation of alpha2-receptors. Two possible explanations could account for these observations. First, within the NPP in fish, activation of alphal- and alpha2-receptors produces the same cellular response, either excitation or inhibition. Alternatively, both receptor types could follow the mammalian pattern, with occupation of alpha2-receptors inhibiting heat gain pathways, and occupation of alphal-receptors activating heat loss pathways. An inhibitory role for NE in the thermoregulatory centers of endothermic vertebrates has also been suggested (Zeisberger and Bruck 1976).

In comparison to NE, the agonists require doses three to four orders of magnitude higher to obtain the same thermoregulatory effect. A possible explanation for this is that more than one adrenergic receptor class may be involved in the thermoregulatory effect of NE. Clondine, while a potent alpha2 agonist, has been shown to be a partial alphal agonist (Ruffolo et al. 1980). Hence, the high potency of clonidine, relative to phenylephrine, may be due to complete activation of alpha2 and partial activation of alphal receptors. In the cat, higher doses of phenylephrine are also required to obtain an effect equivalent to that of NE (Rudy and Wolf 1971).

Most studies on the involvement of adrenoreceptors in thermoregulation have been done on endothermic vertebrates.

Central injections of alpha-adrenergic antagonists, but not
beta-adrenergic antagonists, can block the effect of NE in
cats (Burks 1972), rats (Fukushima and Itoh 1975), and
pigeons (Chawla et al. 1974). In other species, including
chickens (Nistico et al. 1976) and primates (Mora et al.
1983), the alpha-receptor class still predominates, but a
minor beta component also appears to exit. We observed a
minor attenuation of the decrease in selected temperature
with a beta blocker. However, this attenuation was not
significant, nor was the activation seen with the beta
agonist. Hence, the effects observed for the
beta-adrenorecepters may be due to interactions with
non-thermoregulatory centers in the brain.

In endothermic vertebrates, the injection of
noradrenergic antagonists by themselves have shown variable
results. In the pigeon, phenoxybenzamine, an
alpha-antagonist, produced a small but consistant,
long-lasting rise in body temperature, while propranolol
produced a nonspecific decrease in body temperature (Chawla
et al. 1974). On the other hand, in the cat, an
alpha-antagonist produced inconsistent increases in body
temperature (Felberg and Saxena 1971), while in the rat, no
effect on body temperature was observed (Gisolfi and
Christman 1980). In the present study, the injections of
either phentolamine or propranolol by themselves had no
effect on thermoregulatoy behavior. This suggests that NE

may not exert continuous influences on thermoregulatory centers in fish.

The precise role of noradrenergic input to the neurons regulating temperature selection in fish remains unclear. One alternative is the possible participation in cold acclimation. In the present study, fish were maintained at 25 C, the approximate temperature selected by goldfish left in a gradient for a long period of time (Coutant 1977). However, following acclimation to lower temperatures (e.g., 7 C) for several weeks, goldfish, when placed in a thermal gradient, select about 13 C and then gradually select higher temperatures. After about 24 hours, they are again selecting temperatures near 25 C (Crawshaw 1980). Noradrenergic systems may be involved in the lowering of selected temperatures that occurs during cold acclimation.

CHAPTER VI

CONCLUSION

NE appears to be involved in the hypothalamic circuit mediating the selection of cooler water. The site is located in the anterior NPP. This decrease in selected temperature appears to be mediated by alpha-adrenoreceptors, with both an alpha1 and alpha2 component. Since antagonists injected by themselves do not have a thermoregulatory effect, NE may not have a role in the short term regulation of body temperature in fish but rather may play a role in the response to long term changes or extreme thermal conditions. In fish, the exact role that the anterior NPP and particular neurotransmitters play within the thermoregulatory network await further experimentation.

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