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CYTOKINE MODULATION OF DEFENSIVE RAGE BEHAVIOR IN THE CAT: ROLE OF $GABA_A$ AND INTERLEUKIN-2 RECEPTORS IN THE MEDIAL HYPOTHALAMUS

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Abstract—Defensive rage behavior is a form of aggressive behavior occurring in nature in response to a threatening stimulus. It is also elicited by stimulation of the medial hypothalamus and midbrain periaqueductal gray (PAG) and mediated through specific neurotransmitter-receptor mechanisms within these regions. Since interleukin (IL)-2 modulates the release of neurotransmitters linked to aggression and rage, we sought to determine whether IL-2 microinjected into the medial hypothalamus would modulate defensive rage. Microinjections of relatively low doses of IL-2 into the medial hypothalamus significantly suppressed defensive rage elicited from the PAG in a dose-dependent manner and in the absence of signs of sickness behavior. Pre-treatment with an antibody directed against IL-2Rα or a $GABA_A$ receptor antagonist blocked IL-2's suppressive effects upon defensive rage. Since the suppression of defensive rage is also mediated by $5\text{-}HT_1$ receptors in the medial hypothalamus, a $5\text{-}HT_1$ antagonist was microinjected into this region as a pretreatment for IL-2; however, it did not block IL-2's suppressive effects. Immunocytochemical data provided anatomical support for these findings by revealing extensive labeling of IL-2Rα on neurons in the medial hypothalamus. IL-2 microinjected into the medial hypothalamus did not modulate predatory attack elicited from the lateral hypothalamus. In summary, we provide evidence for a novel role for IL-2 in the medial hypothalamus as a potent suppressor of defensive rage behavior. These effects are mediated through an IL-2-$GABA_A$ receptor mechanism. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Interleukin (IL)-2 is a pleiotropic cytokine that potently modulates CNS activity. IL-2 mRNA, protein and receptor genes have been detected on neurons and glia in a variety of brain regions, notably the hypothalamus (including medial hypothalamus), hippocampus, septal area, cortex, and striatum (Araujo et al., 1989; Eizenberg et al., 1995; Hanisch and Quirion, 1995; Petitto and Huang, 1994; Korneva et al., 2000).

Functionally, IL-2 influences central GABA-ergic and glutamatergic transmission (Rozsa et al., 1997; Seto et al., 1997; Ye et al., 2001). IL-2 also induces cytokine-specific alterations of monoamine, neuroendocrine and unit activity in the limbic–hypothalamic–midbrain axis, a region typically associated with the expression of emotional forms of behavior (Zalcman et al., 1994; Anisman et al., 1996; Pauli et al., 1998; Karanth et al., 1993; Bartholomew and Hoffman, 1993; Korneva et al., 2000; see Hanisch and Quirion, 1995; Dunn, 2001). In addition to modulating neurotransmitter and neuroendocrine activity, IL-2 influences the individual's behavioral response to potentially threatening environmental stimuli (Nistico and De Sarro, 1991; Petitto et al., 1997; Zalcman et al., 1998; Lacosta et al., 1999; Zalcman, 2001, 2002). For example, IL-2 causes an increase in novelty-induced exploratory activity and locomotion (Nistico and De Sarro, 1991; Petitto and Huang, 1994; Zalcman et al., 1998; Zalcman, 2001, 2002). It is important to note, however, that most studies assessing the behavioral effects of IL-2 involved peripheral administration of the cytokine. Although IL-2 potently modulates central neurotransmitter activity, very little is known about the behavioral consequences of microinjections of IL-2 into specific regions of the brain, or how cytokine-receptor interactions may mediate such effects.

Since IL-2 is present in the hypothalamus and influences neurotransmitter functions, the question may be raised whether IL-2 plays a role in the regulation of functions associated with the hypothalamus. One such behavior associated with the medial hypothalamus is a form of aggression referred to as defensive rage, which is typically studied in the cat. It is characterized by marked hissing, striking of another animal of the same or different species, arching of the back, piloerection, retraction of the ears, and extension of its claws (Flynn, 1967; Siegel and Brutus, 1990; Siegel et al., 1999). The significance of this model is underscored by the fact that this form of aggressive behavior occurs in response to a real or perceived threat within an animal's environment (Leyhausen, 1979). Because this response involves a reciprocal anatomical and functional relationship between the medial hypothalamus and midbrain periaqueductal gray (PAG), it can reliably be elicited from both of these regions over a period of weeks and even months.

As a result of these features, this model has been used effectively for the study and analysis of the anatomical substrates and neurotransmitter-receptor mechanisms within the

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Abbreviations: IHC, immunohistochemistry; IL, interleukin; IL-2Rα, interleukin-2 receptor α subunit; PAG, periaqueductal gray.

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PAG and hypothalamus that regulate defensive rage behavior (Siegel and Pott, 1988; Siegel et al., 1999; Gregg and Siegel, 2001). These studies have revealed that the different monoaminergic receptors (Barrett et al., 1990; Hassanain et al., 2003a; Shaikh et al., 1997; Sweidan et al., 1990, 1991) play significant roles in the modulation of defensive rage behavior in the cat. Specifically, activation of 5-HT_1 receptors suppresses, while 5-HT_2 receptors facilitate defensive rage (Shaikh et al., 1997; Hassanain et al., 2003a). Activation of other monoamine receptors, including noradrenergic α-2 and dopamine D_2 receptors facilitate this form of aggression (Barrett et al., 1990; Sweidan et al., 1991). Likewise, other studies have further demonstrated that substance P-NK_1, cholecystokinin-B, and NMDA receptor activation facilitates defensive rage, while $GABA_A$ receptor activation powerfully suppresses this form of aggression within the hypothalamus or PAG (Bhatt et al., 2003; Cheu and Siegel, 1998; Han et al., 1996a,b; Lu et al., 1992; Luo et al., 1997; Schubert et al., 1996b).

Most recently, our laboratory has demonstrated that microinjections of relatively low doses of IL-1 into the medial hypothalamus potently facilitate feline defensive rage behavior elicited from the PAG (Hassanain et al., 2003b). It was further shown that this effect is mediated via a 5-HT_2 receptor mechanism. In contrast, IL-6, a cytokine that also influences central serotonin activity (Zalcman et al., 1994; Wang and Dunn, 1998), had no effect upon the rage response when microinjected into the same sites (Hassanain et al., 2003b), indicating that cytokine-induced effects upon defensive rage behavior are highly specific.

In view of the fact that IL-2 in the CNS can induce neurotransmitter release, and, in particular, neurotransmitters known to modulate aggression and rage, we sought to determine whether IL-2 microinjected into the medial hypothalamus could modulate defensive rage behavior, and whether such effects are mediated through IL-2 receptors. Since preliminary evidence from our laboratory had suggested that IL-2 suppresses defensive rage, we further attempted to determine if IL-2's effects are mediated through $GABA_A$ or 5-HT_{1A} receptors, which have also been shown to suppress this form of aggression.

EXPERIMENTAL PROCEDURES

Animals and surgical manipulations

A total of 11 adult female cats weighing between 2 and 4 kg (Liberty Laboratories, Waverly, NY, USA) were employed in the experiments of the present study. All animals were housed individually in their home cages and were maintained on an *ad libitum* feeding and drinking schedule. All experiments were approved by the Institutional Animal Care and Use Committee of New Jersey Medical School (Newark, NJ, USA). All experiments conformed to the guidelines on the ethical treatment of animals as described by the Institutional Animal Care and Use Committee and National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize animal suffering and the number of cats required for each experiment. During aseptic surgery, cats were deeply anesthetized with isoflurane (1–2%). Twenty-four stainless steel guide tubes (17 gauge, 10 mm in length) were stereotaxically mounted bilaterally over holes drilled through the skull overlying the medial hypothalamus, lateral hypothalamus and dorsal midbrain PAG (according to the atlas of Jasper and Ajmone-Marsan, 1954) and were cemented using dental acrylic (Lang Dental Mfg. Co., Wheeling, IL, USA). After that guide tubes were filled with bone wax (Ethicon Inc, Somerset, NJ, USA). Three stainless steel stylets attached to the skull served as an indifferent electrode. One steel bolt was placed into a hole drilled into the nasal sinus of the cat, and two nylon bolts were anchored to the skull with dental acrylic cement. A plastic safety cap was then secured to these bolts to protect the entire assembly and subsequently implanted electrodes from damage.

Experimental model and stimulation parameters

Four strengths are associated with these models of aggressive behavior. First, the responses elicited by brain stimulation closely mimic the aggressive behavior exhibited under natural environmental conditions. Second, these responses can be elicited easily by electrical stimulation of the hypothalamus or PAG in a repeated manner over a given experimental session. Third, these responses are capable of being quantified by measuring current threshold or latency. Fourth, the responses are stable, thus permitting systematic examination of how such forms of aggression can be modified by physiological or pharmacological challenge to the animal, or by changes in environmental conditions. In previous studies, our laboratory utilized these models to examine: the effects of temporal lobe seizures (Brutus et al., 1986), systemic (Shaikh et al., 1987; Sweidan et al., 1990) as well as local intracerebral administration of drugs (Bhatt et al., 2003; Gregg and Siegel, 2003; Hassanain et al., 2003a,b; Sweidan et al., 1991), and the effects of systemic ethanol administration (Han et al., 1997; Schubert et al., 1996a) upon each of these forms of aggressive behavior.

The procedures used for eliciting defensive rage behavior were identical to those used previously in our laboratory as indicated above. Experiments were carried out in awake cats. Cats were freely moving except for the brief period of drug delivery and electrode implantation when the cat was placed in a restraining cat bag under a head holder.

Electrodes were lowered at least 2 weeks after surgery. A stimulating electrode was lowered into the midbrain PAG and a cannula electrode was lowered into the medial hypothalamus. Electrodes were lowered through guide tubes implanted overlying either the medial hypothalamus for defensive rage or PAG in 0.5 mm increments and stimulation was applied at each increment. Once a defensive rage site was located, the monopolar- (or cannula) electrode was cemented in place using dental acrylic. Stimulation consisted of biphasic, rectangular electrical pulses (0.2–0.8 mA, 63 Hz, 1 ms per half cycle duration). Electrical stimulation was generated by grass S-88 stimulators connected through differential amplifiers (Tektronix ADA400A, Richardson, TX, USA) to the cat. A Tektronix TDS 3012 digital oscilloscope was used to monitor peak to peak current.

Measurement of aggressive responses

The response latency for defensive rage was defined as the time required for the cat to hiss after the onset of electrical stimulation. The hissing response was used as the principal measure of defensive rage since this component of the overall response is elicited on each trial (Bhatt et al., 2003). For any given experimental session, stimulation parameters were kept constant. The duration of stimulation was limited to 15 s on all trials. If a response could not be elicited within 15 s, a response latency score of 15 s was recorded even though stimulation was ineffective in generating defensive rage (or predatory attack, see below). If a response was elicited within 15 s, stimulation was terminated and the response latency was recorded. Current delivered to the animals was adjusted to levels that would induce hissing responses at latencies that would permit a determination of suppressing as well

as facilitating effects of drug administration. Accordingly, adjusted currents did not induce immediate responses which would have otherwise prevented analysis of potential facilitating effects of the drug.

Dual stimulation

An initial procedure was conducted utilizing a dual stimulation paradigm involving the medial hypothalamus and PAG, to identify sites in the medial hypothalamus at which stimulation could be shown to facilitate the occurrence of defensive rage behavior elicited from the PAG. This approach was used to ensure that there was a functional relationship between the hypothalamic and PAG attack sites in each cat. In order to identify sites in the medial hypothalamus that could be used for modulation, an electrode was implanted from which hissing could be elicited by stimulation. Then, a dual stimulation technique was applied, during which either the PAG was stimulated alone or the PAG and hypothalamus were stimulated concurrently (at 63 Hz) with a 4 ms delay separating the biphasic pulses delivered to each region. The current applied to the modulating site in the hypothalamus was maintained at a level below threshold for elicitation of hissing. In this phase of the study, 10 paired trials of single stimulation of the PAG and dual stimulation of the PAG and medial hypothalamus were administered in an A-B-B-A fashion in which 'A' represented stimulation of the PAG alone and 'B' dual stimulation of the PAG plus medial hypothalamus. Response latencies for hissing were determined for each trial. Paired trials of single stimulation were compared with those of dual stimulation by a *t*-test for paired observations. If the *t*-test was significant ($P<0.05$), the hypothalamic site was designated as one that produced modulation. In all cases, it was found that when hissing was elicited from a hypothalamic electrode, it also facilitated PAG-elicited hissing.

Drugs and drug administration

In the next stage of this investigation, we sought to determine the effects of selective doses of IL-2, anti-human IL-2 monoclonal antibody (PeproTech Inc., Rocky Hill, NJ, USA), and anti-human IL-2 receptor α (IL-2Rα) neutralizing antibody (R&D Systems, Minneapolis, MN, USA) upon defensive rage. The $GABA_A$ receptor antagonist bicuculline (Sigma, St. Louis, MO, USA), the 5-HT_{1A} antagonist p-MPPI (Sigma), the antibody directed against IL-2Rα, and the 5-HT_{1A} receptor antagonist were microinjected through a 0.5 μl microsyringe (SGE, Austin, TX, USA) into the medial hypothalamic site from which defensive rage could be elicited. The drug doses were determined empirically from pilot experiments. The order of drug delivery was randomized. On the day in which a drug or vehicle was delivered, five trials of single stimulation of the PAG, at 2 min intervals between stimulation trials were initially administered prior to drug delivery. Since the same site was tested multiple times against different levels of drug with and without pretreatment, the efficacy of that site was tested after every four to five microinjections by dual stimulation in order to demonstrate that modulatory effects of dual stimulation were still present. In addition, the last experiments conducted for each animal included a comparison of the effects of pretreatment with antibody against administration of IL-2 plus vehicle. In each of these instances, the hissing response was clearly present, indicating the continued efficacy of the sites in question.

For drug delivery, the cat was first restrained in a veterinary restraining bag. Then, the plastic head cap was removed and the head was gently placed in a brass head holder on a stereotaxic apparatus using the three bolts mounted on its head during surgery. Cats experienced no stress or discomfort following gradual habituation to the apparatus over the course of several days. This was evidenced by the observations that animals typically purred or were quiet during this period and made no attempts at escaping the restraining bag. After the cat was placed in the head holder, the 0.5 μl microsyringe was lowered through the cannula electrode into the medial hypothalamus and placed 0.5 mm below the electrode tip. The drug or saline was injected over a period of 2 min. After injection, the syringe was left in place for 1 min to allow for diffusion and was removed slowly. For experiments in which both IL-2 and (a) anti-IL-2 monoclonal antibody, (b) anti-IL-2 receptor α antibody, or (c) GABA or serotonin antagonist were administered, the total injection volume was 0.5 μl, delivered with a delay of 5 min between injections. In order to test the specificity of IL-2 upon defensive rage, an anti-human IL-2 monoclonal antibody was administered into the medial hypothalamus prior to delivery of IL-2 into that site. The cat was then removed from the head holder and returned to the experimental chamber. The cat was given five trials of stimulation over each of the following four blocks of time: 30–40, 60–70, 120–130 and 180–190 min post-injection, with an average inter-trial interval of 2 min. The order of treatments was randomly determined.

The design of experiments involved the following stages: (a) a dose-response experiment which included administration of three doses of IL-2, and saline; studies of the effects of: (b) an anti-IL-2 monoclonal antibody; (c) an antibody directed against the IL-2 receptor α subunit (IL-2Rα); (d) the $GABA_A$ receptor antagonist, bicuculline methiodide (Sigma); and (e) the 5-HT_{1A} receptor antagonist, p-MPPI (Sigma).

Behavioral and site specificity of IL-2

To assess the behavioral specificity of IL-2, its effects were investigated on another form of aggression in cats, predatory attack behavior. Predatory attack behavior is characterized by initial stalking of the prey object (i.e. an anesthetized rat), which is followed by biting of the back of its neck or back of the rat (Wasman and Flynn, 1962). Besides mild pupillary dilation, other overt signs of autonomic involvement are not apparent in this response. The methods for inducing predatory attack in the laboratory have been published previously (Bhatt et al., 2003; Han et al., 1997) and the methods used in the present study for the analysis of this form of aggression were identical to those described for defensive rage behavior.

Site specificity of IL-2

The next experiment sought to determine whether the effects of IL-2 on defensive rage were site-specific. Accordingly, IL-2 was injected into the lateral hypothalamus from which predatory attack behavior was elicited and its effects upon defensive rage elicited from the PAG were determined.

Pyrogenic effects of IL-2

To determine the possible pyrogenic effects of administration of the highest dose of IL-2 (5 ng) into the medial hypothalamus, rectal body temperature for each cat was measured at the same pre- and post-injection time periods as described above.

Histology

Cats were perfused transcardially with 0.9% saline (pH 7.2) followed by 4% paraformaldehyde (pH 7.4) at 4 °C. Brains were removed from the skull, blocked, and placed in 4% paraformaldehyde solution at 4 °C for overnight. Then, they were transferred to 30% sucrose solution at 4 °C. Brain sections were cut in a cryostat (Leica CM1900) at 20–25 μm at −20 °C. Sections were mounted on gelatin-coated slides, air dried and stored at −20 °C until processed for immunohistochemistry (IHC).

IHC

Sections were stained for the distribution of IL-2 receptors in the medial hypothalamus, using methods for antigen retrieval and for

determining anti-peroxidase activity. For antigen retrieval, sections were immersed in 0.01 M citric acid (pH 6.0) buffer and heated for 10 min at 95 °C. Sections were washed and treated with 0.3% H_2O_2 for 5 min for anti-peroxidase activity. Sections were washed and blocked with blocking buffer (2% BSA, 2% goat serum, 0.3% Triton X-100 in PBS, pH 7.4) for 1 h. They were then incubated overnight with the primary antibody directed against the IL-2Rα (15 μg/ml of murine anti-human IL-2Rα antibody, R&D Systems) at 4 °C in a moisturizing chamber. Primary antibody dilutions were carried out in blocking buffer. Sections were washed 3×5 min and then incubated with 1:333 dilution of the secondary antibody to the IL-2 receptor (biotinylated goat–anti-mouse; Santa Cruz Biotechnology, Santa Cruz, CA, USA), for 2 h at RT in a moisturizing chamber. Sections were then washed and treated with avidin–biotin complex (ABC kit; Vector Laboratories, Burlingame, CA, USA) for 45 min and then treated with diaminobenzidine for color development. Slides were further washed with distilled water and dehydrated with alcohol and xylene. Photomicrographs of sections were taken with an Olympus AX-70 microscope using a Magnafire digital camera at 10× and 20× objective lens (Olympus, Melville, NY, USA).

Omission controls were processed simultaneously, where pre-selected slides were omitted for incubation with the primary antibody, while all other steps were identical.

Statistical analysis

Modulation of PAG-elicited defensive rage by the medial hypothalamus was determined by a *t*-test for paired observations comparing latencies between paired trials of single (PAG alone) and dual stimulation of the PAG and medial hypothalamus. The remaining statistical analyses were based upon transformed data that were changed from response latency scores to percentage change in response latencies relative to baseline response latencies. The percentage change was calculated as follows: percentage change=[(post-drug latency−pre-drug latency)/pre-drug latency]×100. This calculation was applied so as to eliminate differences between animals with respect to pre-injection baseline latencies.

A two-way randomized blocks ANOVA was used to analyze the effects of different doses of drugs (variable A) upon response latencies over the pre-injection and four post-injection time periods (variable B). A Newman-Keuls multiple comparisons test was employed to determine the difference in percent changes in responses at different epochs of time, post-injection ($P<0.05$).

RESULTS

Anatomical localization of electrode tips

The distribution of anatomical sites within the PAG from which stimulation induced defensive rage behavior, and the medial and lateral hypothalamus from which stimulation or microinjections of IL-2 and/or GABA compounds were applied to defensive rage and predatory attack sites, respectively, are illustrated in Fig. 1. Sites from which stimulation elicited defensive rage were limited mainly to the dorsolateral aspect of the middle third of the PAG. Cannula electrodes associated with defensive rage were situated mainly in the anteromedial and dorsomedial hypothalamus. Cannula electrodes associated with predatory attack were located in the middle third of the lateral hypothalamus.

Medial hypothalamic facilitation of defensive rage elicited from the PAG

We sought to determine the functional relationship between the PAG and the medial hypothalamus in order to confirm the integrity of the circuit linking these two sites.The results shown in Fig. 2 demonstrated that dual stimulation of the medial hypothalamus facilitated PAG-elicited defensive rage in each of the cats tested ($P<0.05$). The average reduction in response latencies following dual stimulation was 70% (range 47%–84%), thus demonstrating the existence of a functional relationship between the PAG and medial hypothalamus sites used in this study.

Effects of microinjections of selective doses of IL-2 on defensive rage

The next phase of this study sought to determine the effects of IL-2 microinjections into the medial hypothalamus upon defensive rage behavior elicited from the PAG. The results indicated that injection of IL-2 into sites within the medial hypothalamus that facilitated defensive rage induced a significant suppression of hissing in a time and dose dependent manner (Fig. 3A). In a two-way ANOVA where three doses of IL-2 (50 pg, 500 pg and 5 ng) and saline were tested over five blocks of time (pre-injection, 30–40 min, 60–70 min, 120–130 min, 180–190 min), a significant suppression of the hiss response [$F(3,9)=13$, $P<0.001$] was observed (Fig. 3, upper panel). A Newman-Keuls multiple comparisons test of the main effect of Dose showed that 5 ng of IL-2 induced a significant suppression of the hiss response compared with 50 pg, 500 pg, and saline. The maximal suppression induced by 5 ng of IL-2 was observed at 60 min (192%), declined over time, and returned to baseline levels at 180 min. At 120 min post-injection, 500 pg of IL-2 also induced a significant suppression of the hiss response relative to that seen with either 50 pg or saline ($P<0.01$), and returned to baseline at 180 min. At other epochs of time, 50 pg of IL-2 did not significantly alter response latencies. Taken together, these data suggest that IL-2 potently suppressed defensive rage behavior.

To test whether injection volume was a factor in this study, a separate experiment was conducted to compare the effects of 0.25 μl of IL-2 alone to 0.25 μl saline+0.25 μl IL-2. The results revealed no significant differences between these two volumes at all the time blocks tested ($P>0.05$).

Microinjections of IL-2 and body temperature

Rectal temperature measured over the epochs of time following microinjections of the dose of IL-2 that induced maximal suppression of hissing failed to alter body temperature [$F(4,20)=0.04$, $P=0.99$] (Fig. 3, lower panel). Moreover, casual observations of the animals following microinjections revealed no signs of lethargy, discomfort or loss of appetite. Hence, the suppressive effects of IL-2 cannot be attributed to sickness behavior.

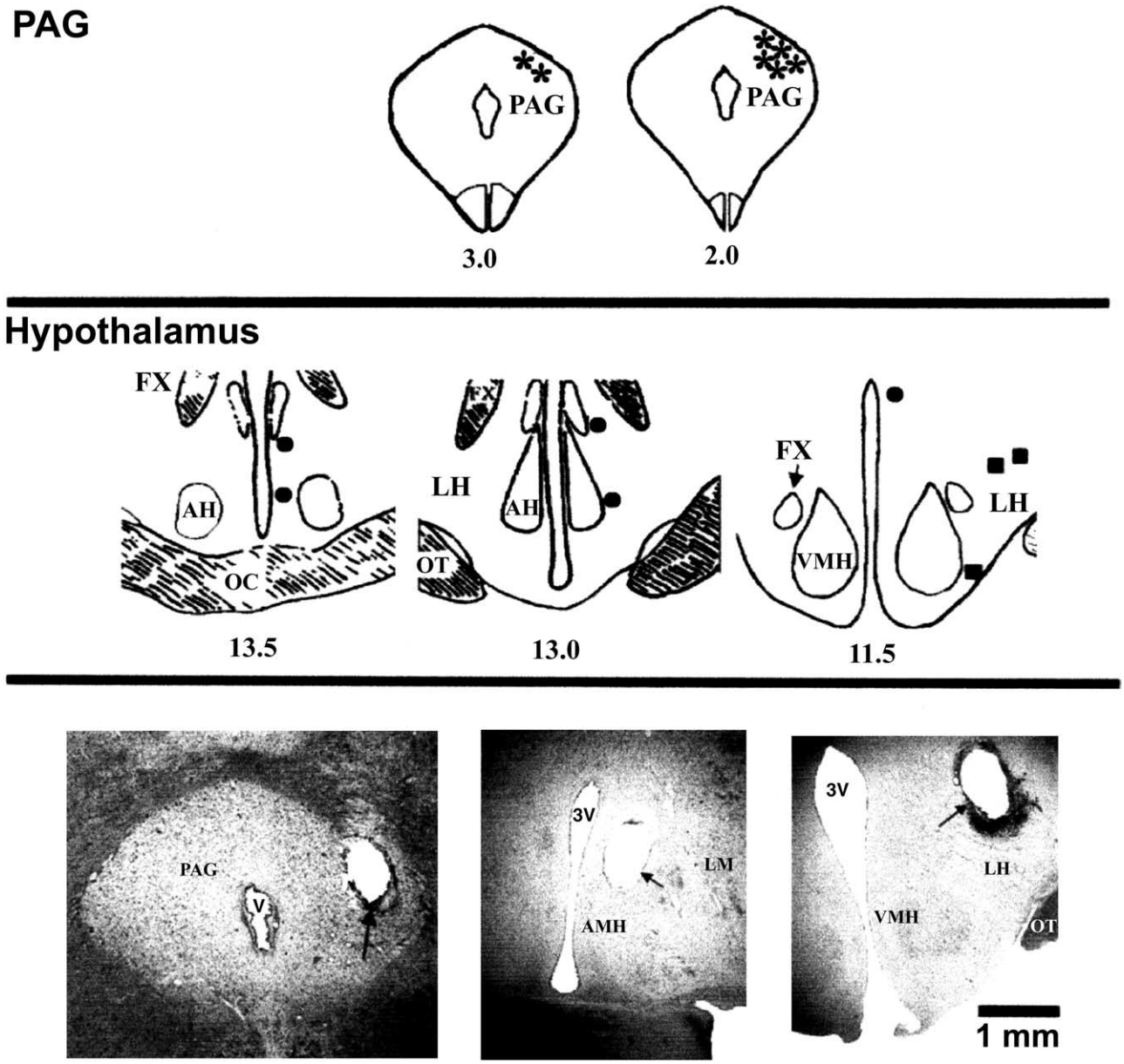

Fig. 1. Stimulation and injection sites. Location of tips of electrodes used to elicit defensive rage from the PAG (stars; upper panel). Tips of cannula electrodes used for microinjections into the medial hypothalamus (filled circles; middle panel). Tips of electrodes used to elicit predatory attack from the lateral hypothalamus (filled squares; middle panel). Sections showing the presence of electrode tips in the PAG, medial hypothalamus and lateral hypothalamus (lower panel). AH, anterior hypothalamus; AMH, anterior MH; FX, fornix; LH, lateral hypothalamus; OC, optic chiasm; OT, optic tract; VMH, ventromedial hypothalamus; 3v, third ventricle.

The effects induced by IL-2 are blocked by pre treatment with anti-IL-2 antibody

The results indicated that pre-treatment with anti-IL-2 antibody completely blocked the suppressive effects of IL-2 upon hissing for up to 120 min post-injection [$F(1,3)=18.03$, $P<0.01$] (Fig. 4). The anti-IL-2 antibody administered alone had no significant effect upon the response latency ($P>0.05$).

Pretreatment with an IL-2 receptor antibody (anti-IL-2Rα) blocked the IL-2-induced effects on defensive rage behavior

To determine if the effects of IL-2 are mediated by the IL-2 receptor, an antibody directed against the IL-2Rα was microinjected into the same hypothalamic site 5 min prior to IL-2 microinjection. Pre-treatment with an anti-IL-2Rα antibody blocked the suppressive effects of IL-2 upon PAG-elicited

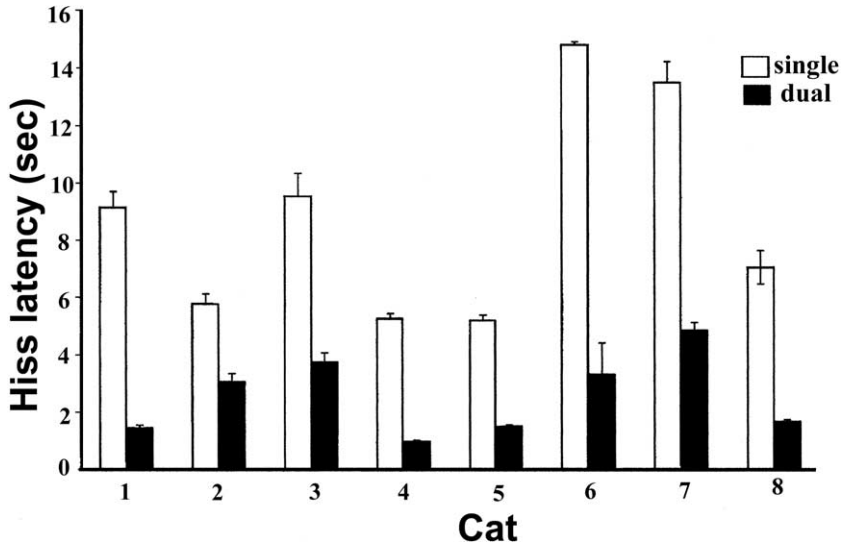

Fig. 2. Dual and single stimulation: defensive rage behavior elicited from the PAG was facilitated by dual stimulation in each of the cats tested ($P<0.05$).

hissing [$F(1,3)=10.89$, $P<0.01$] (Fig. 5). Microinjection of the anti-IL-2Rα antibody alone had no significant effect upon response latencies [$F(3,86)=0.43$, $P=0.73$] (Fig. 5).

Suppression of defensive rage by IL-2 is mediated by $GABA_A$ receptor

To determine whether IL-2 suppression of defensive rage is mediated through $GABA_A$ receptors, the IL-2 site in the medial hypothalamus was pretreated with bicuculline. The results showed that the $GABA_A$ receptor antagonist blocked the suppressive effects induced by IL-2 [$F(1,3)=15.82$, $P<0.01$] (Fig. 6, upper panel). Microinjection of bicuculline alone had no effect upon response latencies for hissing over time [$F(3,96)=0.10$, $P=0.96$]. Similar observations for this dose level were obtained previously in our laboratory (Cheu and Siegel, 1998).

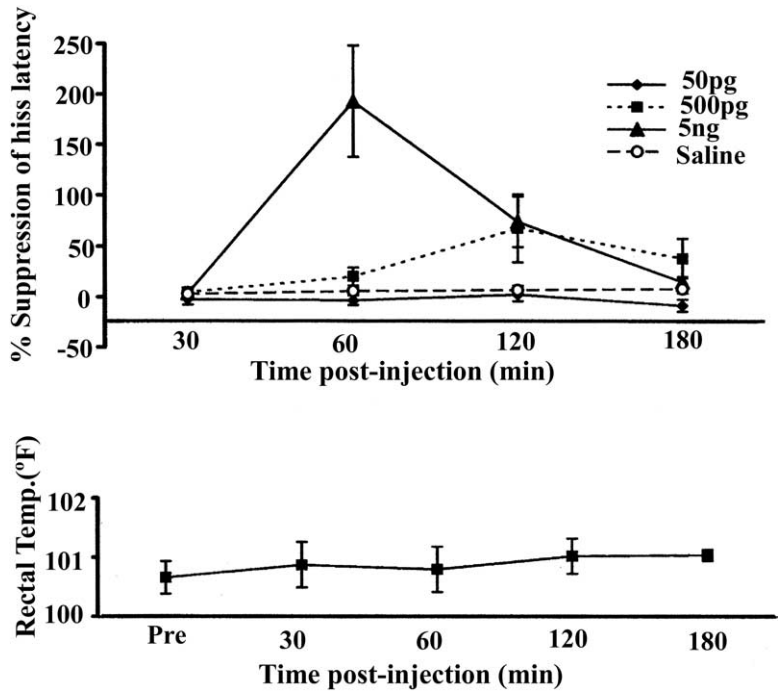

Fig. 3. Effects of microinjections of IL-2 into the medial hypothalamus upon defensive rage. IL-2 dose-dependently suppressed defensive rage elicited from the PAG in a dose- and time-dependent manner ($P<0.001$). Maximal suppression of defensive rage was induced by 5 ng of IL-2 (upper panel). Microinjections of 5 ng of IL-2 had no effect on rectal temperature (lower panel).

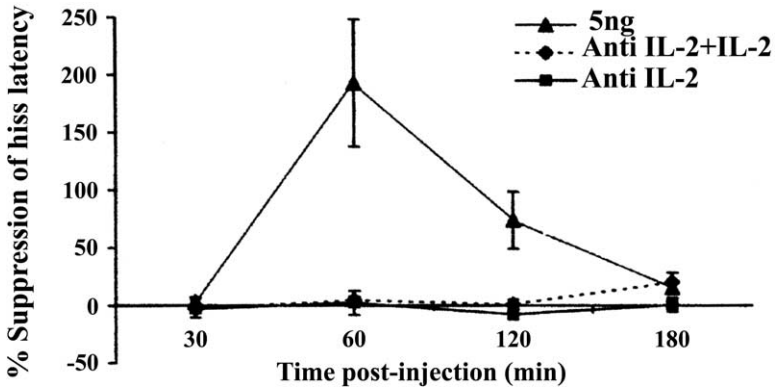

Fig. 4. Effects of an anti-IL-2 monoclonal antibody upon defensive rage. Pre-treatment with an anti-IL-2 monoclonal antibody blocked the suppressive effects of IL-2 upon PAG-elicited defensive rage ($P<0.01$).

Pretreatment with the 5-HT_{1A} receptor antagonist, p-MMPI

To study the possible role of 5-HT_{1A} receptors in IL-2-mediated suppression of defensive rage, the 5-HT_{1A} receptor antagonist p-MPPI was microinjected into the same hypothalamic site 5 min prior to microinjection of IL-2. Microinjections of MPPI had no effect upon the suppressive effects of IL-2 [$F(1,3)=0.08$, $P=0.78$] (Fig. 6, lower panel). Microinjection of MPPI alone had no effect on response latencies [$F(3,96)=0.66$, $P=0.57$].

Microinjections of IL-2 into the lateral hypothalamus upon defensive rage elicited from the PAG

Microinjections of IL-2 into the lateral hypothalamus from which predatory attack behavior could be elicited had no effect upon hiss latencies [$F(3,36)=0.06$, $P<0.98$] (Fig. 7) elicited from stimulation of the PAG. This finding provides evidence that the effects of IL-2 on PAG-elicited defensive rage are specific to sites in the medial hypothalamus associated with defensive rage behavior.

Microinjections of IL-2 into the medial hypothalamus and predatory attack elicited from the lateral hypothalamus

Microinjections of IL-2 into the medial hypothalamus did not significantly affect the latencies for predatory attack elicited from the lateral hypothalamus [$F(3,36)=0.1$, $P=0.96$]. The percent changes in response latencies following IL-2 administration were as follows: 30 min: 3.4%; 60 min: 2.91%; 120 min: 1.66%: 120 min: 0.36%. The results of this experiment thus suggest that the suppressive effects of IL-2 upon defensive rage behavior elicited from the PAG are specific to this form of aggression and not to other forms of aggression.

Immunocytochemical labeling of IL-2

Immunocytochemical observations indicated that IL-2 receptors were distributed widely throughout the medial hypothalamus in a relatively uniform manner. In contrast, the lateral hypothalamus revealed a much weaker pattern of labeling (Fig. 8). When the primary antibody was omitted

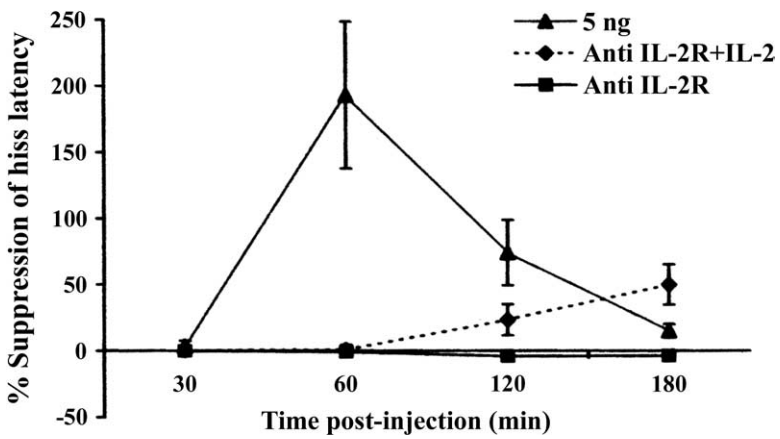

Fig. 5. Effects of an anti-IL-2 receptor antibody (anti-IL-2Rα) upon defensive rage. Pre-treatment with anti-IL-2 receptor antibody blocked the suppressive effects of IL-2 upon PAG-elicited defensive rage defensive rage ($P<0.01$).

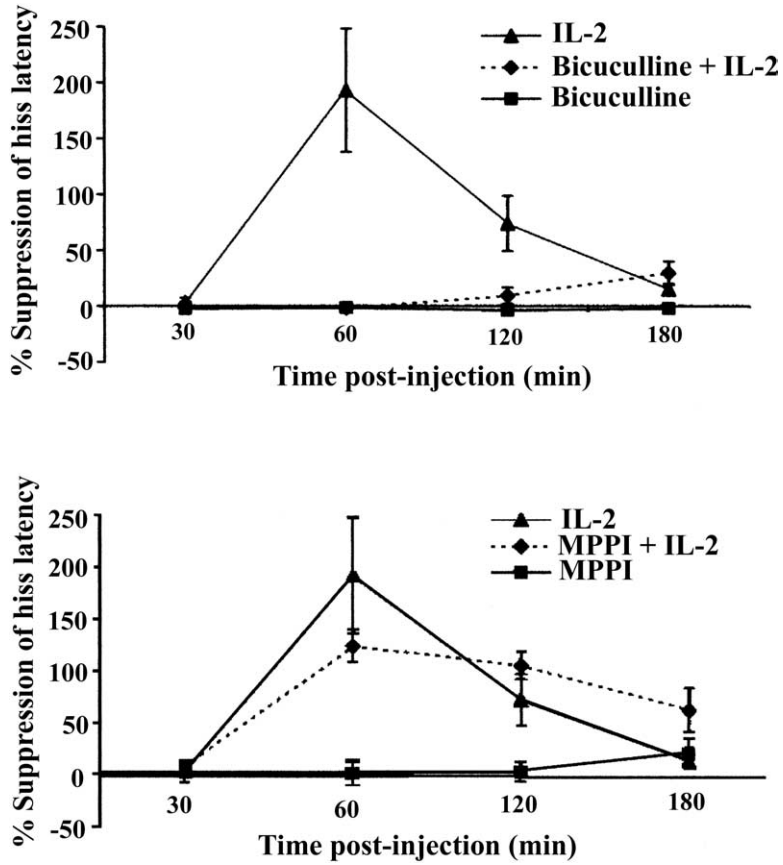

Fig. 6. Effects of a $GABA_A$ receptor antagonist and a 5-HT_{1A} receptor antagonist upon defensive rage. Pre-treatment with the $GABA_A$ receptor antagonist, bicuculline, blocked IL-2's suppressive effects upon PAG-elicited defensive rage ($P<0.01$; upper panel). In contrast, pre-treatment with the 5-HT_{1A} receptor antagonist, p-MMPI, had no effect on IL-2-induced suppression of defensive rage (lower panel).

as a control procedure, no labeling in hypothalamus or elsewhere in the CNS could be detected.

DISCUSSION

In the present investigation, we provide novel evidence that IL-2 in the medial hypothalamus suppresses feline defensive rage behavior, and that this occurs via IL-2 and $GABA_A$ receptor mechanisms. The effect of IL-2 on defensive rage is potent and independent of overt signs of sickness behavior. In contrast with its effects on defensive rage behavior, IL-2 does not influence predatory attack elicited from the lateral hypothalamus. Likewise, the suppressive effects of microinjections of IL-2 were limited to

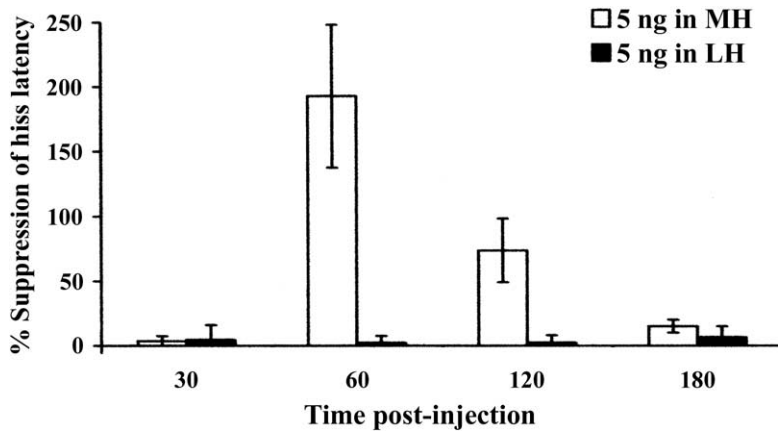

Fig. 7. Site specificity of IL-2's suppressive effects upon defensive rage. Microinjections of IL-2 into the lateral hypothalamus did not affect PAG-elicited defensive rage.

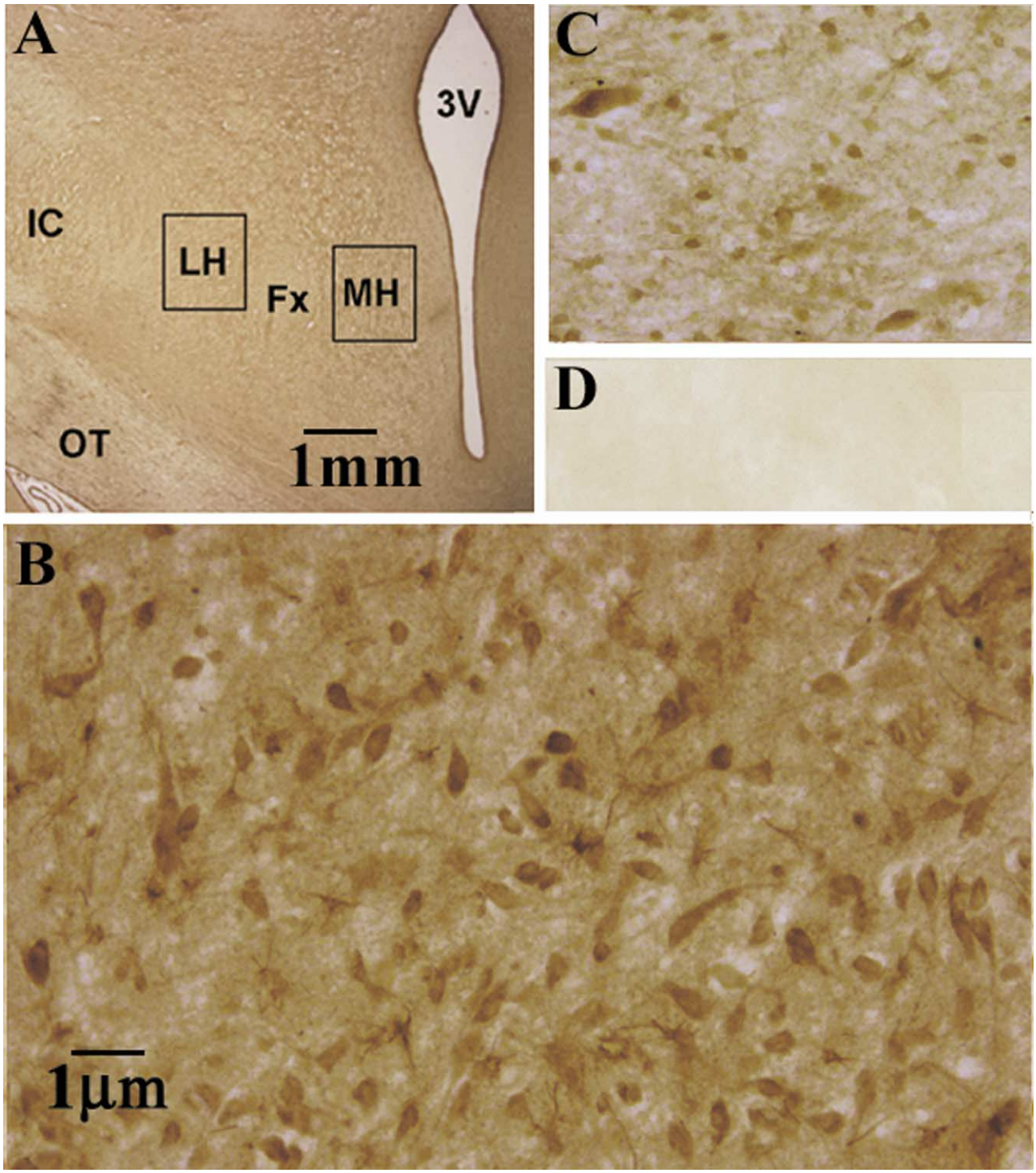

Fig. 8. Immunocytochemistry. Distribution of IL-2 receptors in the medial hypothalamus. (A) Low power view of the anterior hypothalamus indicating the regions from which photomicrographs were taken and shown in panels (B) of the medial hypothalamus, depicting relatively intense and extensive labeling of IL-2 receptors on neurons, and panel (C) of the lateral hypothalamus, depicting relatively sparse labeling on neurons; (D) photomicrograph of a section taken through the medial hypothalamus in which the primary antibody was omitted. Fx, fornix; IC, internal capsule; LH, lateral hypothalamus; MH, medial hypothalamus; OT, optic tract. Scale bars=1 μm, shown in panels B, C, and D.

the medial hypothalamus but did not extend to the lateral hypothalamus. Hence, IL-2 induces site and behavioral selective effects upon defensive rage behavior.

An IL-2 receptor antagonist significantly attenuated the effects of IL-2 on defensive rage behavior. This is the first demonstration that an effect of IL-2 on emotional behavior

is mediated via an IL-2 receptor mechanism. Microinjections alone of the IL-2 receptor antagonist into the medial hypothalamus did not appreciably influence defensive rage behavior, suggesting that its inhibitory effects are not due to non-specific actions of the drug. This finding also suggests that an IL-2 receptor mechanism in the medial hypothalamus does not exert a tonic suppression of defensive rage. While this conclusion may be a reasonable one, it may be limiting in scope because it was derived within the context of a stimulation-based model applied in the present study. The more general question is whether IL-2 is released phasically or tonically in defensive rage behavior occurring under natural conditions. While there is insufficient evidence to allow a precise conclusion to be drawn here, two facts that seem to be relevant to this issue should be noted. The first is that the extensive ethological studies of Leyhausen (1979) have shown very close similarities between defensive rage induced by electrical brain stimulation and defensive rage behavior evoked by a threatening stimulus under natural conditions. This would suggest that similar mechanisms would be present under defensive rage induced under both conditions. Second, a study by Ye et al. (2001) revealed that IL-2 had no effect upon membrane conductance of midbrain dopamine neurons when applied alone; however, IL-2 potently modulated currents directly activated following administration of NMDA. This finding would also appear to support the view that the modulating effects of IL-2 are phasic in nature. Nevertheless, a better understanding of the nature of IL-2's effects upon defensive rage behavior should include studies involving this cytokine within the context of additional models of aggressive behavior.

Although IL-2 receptors have been previously detected in a variety of brain regions, including the hypothalamus (see Hanisch and Quirion, 1995), little is known about IL-2 receptor distribution in the medial hypothalamus. Our present observation of extensive labeling of IL-2Rα on neurons in the medial hypothalamus thus extends previous reports, and provides structural support for our functional findings. From the anatomical immunocytochemical observations in the present study, IL-2 receptors were also observed in the lateral hypothalamus but in relatively smaller quantities (Fig. 8). Since the distribution of brain IL-2 receptors has been previously shown to be similar to that observed for IL-2 mRNA or protein (Lapchak et al., 1991), we suggest that IL-2's modulation of defensive rage involves interactions between IL-2 and IL-2Rα derived from the medial hypothalamus. However, a role for IL-2 released from nerve terminals in the medial hypothalamus should not be discounted. It may be suggested that, under natural conditions, IL-2 is released onto medial hypothalamic neurons, where it may reduce the likelihood of an inappropriate induction of defensive rage behavior.

One could argue that the present effects of IL-2 also involve IL-15 or an IL-15 receptor mechanism since the IL-2/IL-15 receptor system shares the β and γ subunits and certain biological effects of IL-2 are mediated via this receptor system (Giri et al., 1995a,b; Petitto and Huang, 2001). However, we used an IL-2 receptor antibody directed against the non-shared alpha subunit. Although the IL-2 and IL-15 receptor alpha systems are similar but not identical, they exert differential effects on cell signaling (Eicher, 2003). We thus suggest that the present behavioral effects of IL-2 are not associated with an IL-15 receptor mechanism.

IL-2-GABA interactions have been shown to occur in the CNS. For example, $GABA_A$ or $GABA_B$ receptor antagonists block the inhibitory effects of IL-2 on hippocampal acetylcholine release (Seto et al., 1997). IL-2 also reduces GABA-induced inward currents in isolated neurons (Rozsa et al., 1997). Since GABA receptors are present extensively over the hypothalamus (Cheu et al., 1998; Han et al., 1996b), it is likely that $GABA_A$ receptors in the medial hypothalamus lie in proximity to IL-2 receptors. This probable relationship with respect to defensive rage behavior is significant. Previously, it was shown that $GABA_A$ receptors in the medial hypothalamus suppress defensive rage behavior in the cat (Cheu and Siegel, 1998). That similar effects are mediated through activation of IL-2 receptors in the MH and that these effects can be blocked by bicuculline point to the likelihood of the presence of a unique and important mechanism underlying the relationship between IL-2 and $GABA_A$ in the regulation of defensive rage behavior. Since IL-2 receptors are present over the medial hypothalamus and elsewhere in the CNS, it is reasonable to believe that these receptors may interact with the same or different neurotransmitter-receptors in mediating functions associated with the forebrain and brainstem. In support of this notion, preliminary findings obtained from our laboratory suggest that IL-2 receptor activation in the midbrain PAG facilitates, rather than suppresses, defensive rage elicited from the medial hypothalamus (Bhatt et al., 2004). Collectively, these findings coupled with those obtained for IL-2's effects upon predatory attack would imply that the effects of IL-2 on a given behavioral process display a regional and behavioral specificity that may be linked to a distinct interaction between IL-2 and a given neurotransmitter-receptor within the brain region in question. Following this line of reasoning, it may be further suggested that a better understanding of such interactions may best be obtained through an analysis of the molecular signaling pathways associated with IL-2.

Recently, our laboratory has shown that IL-1 is a potent modulator of defensive rage behavior (Hassanain et al., 2003b). When taken together with the present findings that IL-2 in the medial hypothalamus potently suppresses defensive rage elicited from the PAG, it is reasonable to conclude that brain cytokines are important modulators of aggression and rage behavior. Within the medial hypothalamus, there are similarities as well as differences with respect to the effects upon defensive rage that are induced by these two cytokines. Concerning their similarities, the first is that the effects of IL-1 and IL-2 on defensive rage in the medial hypothalamus are induced through relatively low doses of these cytokines. Second, these cytokines do not exert a tonic effect on defensive rage. Third, these effects occur independently of any signs of sickness behavior. However, this does not preclude an adaptive role

for cytokines in influencing the expression of aggressive behavior during an orchestrated or subclinical immune response. It was similarly suggested by Zalcman et al. (1998) that the differential effects of IL-1, IL-2 and IL-6 on exploratory activity could serve adaptive roles during an orchestrated immune response; however, pathological increases or increases of unusually long duration could have severe psychopathological repercussions (see Discussion in Zalcman et al., 1998). Fourth, the effects of IL-1 and IL-2 on defensive rage are mediated via their respective receptors. The primary difference between these two cytokines in the medial hypothalamus is their opposing effects upon defensive rage; namely, that IL-1 facilitates while IL-2 suppresses this form of aggression. Secondly, different cytokine-neurotransmitter actions are involved. Specifically, the facilitatory effects of IL-1 are mediated through 5-HT_2 receptors whereas the effects of IL-2 are mediated through $GABA_A$ receptors. Hence, IL-1 and IL-2 modulate defensive rage behavior in a cytokine-specific manner. This conclusion supports the findings that the effects of IL-1, IL-2 and IL-6 on monoamine activity in the limbic–hypothalamic–midbrain axis and on behavioral responses to a potentially threatening stimulus also occur in a cytokine-specific manner (Zalcman et al., 1994, 1998).

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