ETHANOL ENHANCES MEDIAL AMYGDALOID INDUCED INHIBITION OF PREDATORY ATTACK BEHAVIOUR IN THE CAT: ROLE OF GABA_A RECEPTORS IN THE LATERAL HYPOTHALAMUS

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Abstract — The present study tested the hypothesis that the suppressive effects of ethanol upon predatory attack behaviour in the cat involve a pathway from the medial amygdala to the lateral hypothalamus, and that these suppressive effects are mediated by γ-aminobutyric acid (GABA_A) receptors located in the lateral hypothalamus. Cannula electrodes were implanted into the lateral hypothalamus for elicitation of predatory attack behaviour and for microinjections of the GABA_A receptor antagonist, bicuculline. Monopolar stimulating electrodes were implanted into the medial amygdala from which subseizure levels of electrical stimulation suppressed predatory attack behaviour. In the first phase of the study, we compared response latencies for predatory attack behaviour following single stimulation of the lateral hypothalamus alone with those following paired trials of dual stimulation of the medial amygdala plus lateral hypothalamus. Dual stimulation significantly suppressed predatory attack. In the second phase of the study, peripheral ethanol administration (in doses of 0.01, 0.5 and 1.0g/kg, i.p.) enhanced the suppressive effects of medial amygdaloid stimulation in a dose- and time-dependent manner in which peak effects were obtained 60 min post-injection. In the third phase of the study, bicuculline (0.15 nmol) was microinjected into the lateral hypothalamus both prior to and following paired trials of dual stimulation. Drug infusion blocked the suppressive effects of medial amygdaloid stimulation upon predatory attack behaviour elicited from the lateral hypothalamus, indicating the importance of GABA_A receptors in mediating this suppression. In the fourth phase of the study, bicuculline, microinjected into the lateral hypothalamus at the time when ethanol's effects were maximal (i.e. 60-80 min post-ethanol administration), totally blocked the suppressive effects of ethanol upon medial amygdaloid suppression of this form of aggressive behaviour. In the last phase of the study, bicuculline (0.15 nmol) infusion into the lateral hypothalamus significantly reduced the suppressive effects of ethanol (1.0 g/kg, i.p.) upon predatory attack behaviour elicited from the lateral hypothalamus. These results support the hypothesis that ethanol's suppressive effects upon predatory attack behaviour in the cat are mediated, at least in part, by GABA_A receptors in the lateral hypothalamus. The present and recent findings in our laboratory support the view that GABAergic pathway which arises from the medial hypothalamus whose neurons receive inputs from the medial amygdala.

INTRODUCTION

It is widely thought that ethanol consumption increases the likelihood of aggression and violence in both humans (Pernanen, 1991; Roizen, 1993; Miczek et al., 1994; Zeichner et al., 1994) and animals (MacDonnell et al., 1975; Blanchard et al., 1986, 1987; Winslow et al., 1988; Miczek et al., 1992; Weerts et al., 1993; Miczek et al., 1994). However, our laboratory has recently provided evidence that the directionality of the effects of ethanol administration depends upon the type of aggressive behaviour being examined (Schubert et al., 1996a). Specifically, we found that ethanol administration potentiated the occurrence of defensive rage behaviour elicited by electrical stimulation of the medial hypothalamus, but suppressed predatory attack behaviour elicited by electrical stimulation of the lateral hypothalamus.
Furthermore, we provided evidence that the potentiating effects of ethanol upon defensive rage were mediated over a descending excitatory amino acid pathway from the medial hypothalamus to the midbrain periaqueductal gray matter (PAG) which acts through N-methyl-d-aspartate (NMDA) receptors (Schubert et al., 1996a,b).

Although little is known about the possible neural substrates and mechanisms underlying ethanol suppression of predatory attack behaviour, recently published experiments from our laboratory have provided several clues underlying this phenomenon (Han et al., 1996a,b). These studies demonstrated that the medial amygdala powerfully suppresses predatory attack elicited from the lateral hypothalamus and that this suppression is mediated over a disynaptic pathway from the medial amygdala to the lateral hypothalamus. The first limb of this pathway arises in the medial nucleus of the amygdala and projects to the medial hypothalamus via the stria terminalis. This limb consists of an excitatory, substance P neuron, that acts upon neurokinin NK₁ receptors within the medial hypothalamus. The second limb of this pathway arises in the medial hypothalamus and passes a short distance to the lateral hypothalamus. It consists of an inhibitory, γ-aminobutyric acid (GABA)ergic projection that acts upon GABA_A receptors within the lateral hypothalamus.

An increasing body of literature supports the view that ethanol can alter GABAergic neuronal and cellular processes (Linnoila et al., 1981; Suzdak et al., 1986; Allan and Harris, 1987; Celentano et al., 1988; Mehta and Ticku, 1988; Nutt and Lister, 1988; Buck and Harris, 1990; Givens and Breese, 1990; Lister and Linnoila, 1991; Criswell and Breese, 1993). However, it is not clear from these studies whether ethanol enhances or inhibits GABAergic processes. Considerable evidence indicates that ethanol can enhance GABAergic functions. For example, ethanol administration stimulates GABA-receptor-mediated chloride transport in synaptoneurosomes (Suzdak et al., 1986) and increases muscimol- or GABA-stimulated chloride flux in cerebellar membranes or spinal cord cultures (Allan and Harris, 1987; Celentano et al., 1988; Mehta and Ticku, 1988). Ethanol has also been shown to increase the frequency of action potentials in cerebral cortical neurons (Nestoros, 1980) and to enhance GABA-mediated inhibition of medial septal neurons (Givens and Breese, 1990). However, ethanol can reduce GABA receptor function by decreasing low-affinity GABA binding (Linnoila et al., 1981; Lister and Linnoila, 1991) as well as by decreasing GABA release (Howerton and Collins, 1984) and GABA-mediated cerebellar Purkinje cell discharge (Harris and Sinclair, 1984). It is conceivable that if the effects of ethanol upon GABAergic neurons were dependent upon the specific configuration of the GABAergic receptors in different regions of the central nervous system (Givens and Breese, 1990), then these discrepancies might be resolved.

Although the precise nature of the effects of ethanol upon neuronal function have yet to be elucidated, it is nevertheless clear from these studies that ethanol has a potent modulating effect upon GABAergic neurons. Accordingly, the present study sought to test the hypothesis that GABA_A receptors located in the lateral hypothalamus mediate ethanol enhancement of medial amygdaloid-induced suppression of predatory attack behaviour elicited from the lateral hypothalamus of the cat.

MATERIALS AND METHODS

Subjects

A total of five adult male and female cats weighing between 2.5 and 3.5 kg, purchased from Liberty Laboratories, Waverly, New York, and which did not spontaneously attack rats, were used in this study. All animals were maintained on an ad libitum feeding and drinking schedule throughout the study. All procedures described below were approved by the Institutional Animal Care and Use Committee of the New Jersey Medical School.

Surgical procedures

Cats were fasted overnight and, immediately prior to surgery, they were premedicated, i.m., with a cocktail of 0.27 mg of atropine, 1 mg of acepromazine, and 0.5 mg of butorphanol, which was followed 30 min later with ketamine (25 mg/kg, i.m.). For the duration of surgery, anaesthesia was maintained with 1–2% isoflurane administered through a tracheal cannula. During aseptic surgery, 20 stainless-steel guide tubes (17 gauge, 10 mm in length) were mounted onto holes drilled
bilaterally through the skull overlying the medial amygnda (AP: 11.0–12.5; L: 6.5–7.0) and lateral hypothalamus (AP: 9.5–12.5; L: 2.5) with the aid of the atlas of Jasper and Ajmone-Marsan (1954). These guide tubes enabled electrodes to be accurately lowered at a later time in the awake cat into the lateral hypothalamus from which predatory attack behaviour could be elicited and into the medial amygdala from which stimulation could suppress this response. In addition, a stainless steel stylet was connected by a silver wire to screws mounted in the skull and served as an indifferent electrode. Three bolts were attached to the skull with dental acrylic to hold a plastic cap that served to protect the electrode assembly. The bolts were also used to secure the animal by its attachment to a brass head holder that was connected to a stereotaxic apparatus for micro-injection of drugs at a later time.

Stimulation procedures

After a post-operative period of 1 week, each animal was placed in a wooden observation chamber (61 x 61 x 61 cm) with a deeply anaesthetized rat. Cannula-electrodes were lowered through guide tubes in 0.5 mm steps of the freely moving cat, first into the lateral hypothalamus, in order to identify a site from which predatory attack could be elicited, and then into the medial amygdala from which stimulation could produce suppression of this response (see description below). Electrical stimulation was generated by a Grass S-88 stimulator connected through constant current stimulus isolation units (Grass SIU6) and peak-to-peak current was monitored on a Tektronix 5000 series oscilloscope with differential inputs. Stimuli consisted of biphasic, rectangular pulses (0.25–0.70 mA, 62.5 Hz, 1 ms per half cycle duration). When stimulation produced a predatory attack response that remained stable over a period of 120 min, the electrode was cemented in place with dental acrylic. The latency for predatory attack behaviour was defined as the duration of time from the onset of stimulation to the point when the cat's teeth made contact with the back of the neck of the rat, at which time, stimulation was terminated. If the cat failed to respond within 15 s, stimulation was terminated and a latency score of 15 s was recorded for that trial. At the completion of each experimental session, the apparatus was scrubbed clean and sprayed with Lysol to eliminate odours generated by the cat and rat.

Predatory attack behaviour and experimental paradigm

Predatory attack behaviour is characterized by an initial stalking of the rat, followed by a bite to the back of its neck. This response is elicited by electrical stimulation of the lateral hypothalamus or parts of the brainstem tegmentum (Chi and Flynn, 1971; Berntson, 1973; Proshansky and Bandler, 1975; Shaikh et al., 1987; Siegel and Pott, 1988; Siegel and Brutus, 1990). Figure 1 identifies the sites in the lateral hypothalamus from which predatory attack behaviour was elicited. The procedures utilized to elicit medial amygdaloid suppression of predatory attack are described in detail elsewhere (Han et al. 1996a, b). In brief, a monopolar electrode was lowered through a guide tube and electrical stimulation (0.1 mA, 62.5 Hz, 1 ms per half cycle duration, which was below threshold for elicitation of seizure discharges) was applied at 0.5 mm steps as the electrode was lowered into the amygdala. Paired trials of single (lateral hypothalamus alone) and dual (lateral hypothalamus + medial amygdala) stimulation were delivered in an A-B-B-A paradigm (where A signifies single and B dual stimulation) in order to eliminate order effects. Pulses applied to the hypothalamus were separated from those applied to the amygdala by 4 ms. Response latencies for trials of single and dual stimulation were compared. When stimulation applied to a given site in the medial amygdala produced significant suppression of predatory attack (defined as a 30% increase in response latencies following dual stimulation and P < 0.05 for a paired t-test comparing response latencies for single and dual stimulation), the electrode was cemented in place. Current delivered to both the medial amygdala and lateral hypothalamus was held constant for a given experiment.

The experimental paradigm that was required to assess the effects of ethanol upon medial amygdaloid suppression of predatory attack behaviour included four stages.

Stage 1. This stage was utilized in all of the experiments described below. It consisted of a pre-drug series of five paired trials of single and dual stimulation of the lateral hypothalamus and medial amygdala in order to establish baseline levels of
amygdaloid suppression of attack behaviour prior to drug delivery. Paired trials were separated by 2 min and extended over 20 min.

Stage 2. This stage tested the effects of ethanol administration upon medial amygdaloid suppression of attack. Ethanol was administered i.p. (in doses of 1.0, 0.5 and 0.01 g/kg) and the dual stimulation paradigm, identical to that described for the pre-injection period, was repeated over a 200 min post-injection time period. The post-injection time periods for all experiments at which trials of single and dual stimulation were conducted were as follows: 5–25, 60–80, 120–140 and 180–200 min. The periods of time between blocks of trials were designated as rest periods and consequently, no brain stimulation was conducted during these periods. For stages 1 and 2, a total of five cats were used (n = 5 cats).

Stage 3. This experiment was included in order to establish the precise dose levels of the GABA<sub>A</sub> antagonist, bicuculline, when microinjected directly into the lateral hypothalamus (in doses of 0.15, 0.075 and 0.01 nmol/0.25 μl of 0.9% NaCl, pH = 7.4 for all doses), which block the suppressive effects of medial amygdaloid stimulation. The paradigm for this set of experiments was identical to that described for stage 2 with the exception that bicuculline rather than ethanol was administered to the animal. Following a pre-injection test period, the effects of bicuculline were observed in a 200 min post-injection test period of paired trials of single and dual stimulation. In experiments conducted in stages 2 and 3, the order of delivery of drug was determined in a counterbalanced manner (n = 4 cats).

Stage 4. This experiment was designed to determine whether GABA<sub>A</sub> receptor blockade in the lateral hypothalamus could alter ethanol enhancement of medial amygdaloid suppression of predatory attack behaviour. The paradigm was identical to those described above for stages 2 and 3, with the exception that this experiment included both ethanol and bicuculline administration. Following a pre-drug period of five paired trials of single and dual stimulation, the post-drug test period was carried out in the following manner: initially, the highest effective dose of ethanol, 1.0 g/kg, was given i.p. and the effects of ethanol administration were observed over the next 60 min. Then, bicuculline (0.15 nmol/0.25 μl) was microinjected into the lateral hypothalamus and the effects of drug infusion were determined over a total period of 200 min, post-ethanol administration. Bicuculline was administered 60 min after ethanol administration because the peak effects of ethanol occurred at this point in time (n = 5 cats).

Two additional experiments were conducted. In one experiment, paired trials of single and dual stimulation extended over a 180 min test period in the absence of drug administration in order to demonstrate the relative stability of the suppressive effects of medial amygdaloid stimulation over time (n = 5 cats). In the other experiment, the effects of bicuculline administration (0.15 nmol/0.25 μl) into the lateral hypothalamus upon ethanol (1.0 g/kg, i.p.) suppression of predatory attack elicited from single stimulation of the lateral hypothalamus were determined (n = 5 cats). Here, the experimental procedures were similar to those described above and included administration of the following drug paradigm: (1) ethanol alone, (2) bicuculline + ethanol; and (3) bicuculline alone. In this experiment, only trials of single stimulation of the lateral hypothalamus rather than those of dual stimulation of the lateral hypothalamus and medial amygdala were employed. In addition, the results of this experiment were analysed over a 60 min rather than a 180 min test period.

In a recently completed study in our laboratory, venous blood ethanol concentrations were determined in cats used in the present as well as previous experiments, following i.p. ethanol administration, by a headspace gas chromatographic method (Schubert et al, 1996a). In brief, blood ethanol values reached peak levels of 100.74 mg/dl at 60–80 min, post-injection, which corresponded to the time period when ethanol had its peak effects upon amygdaloid suppression of predatory attack behaviour.

Histology

All animals were perfused transcardially with phosphate-buffered saline (pH 7.4) and fresh paraformaldehyde fixative at 4°C. Sections (40 μm thick) were cut on a freezing microtome for cresyl violet staining. Sections were mounted on gelatin-coated slides, air-dried, and cover-slipped with Permount. These sections were used for identifying the injection sites and electrode tips.
RESULTS

Predatory attack and amygdaloid modulating sites

Histological analysis revealed that the primary region from which predatory attack behaviour was elicited was the rostral half of the perifornical lateral hypothalamus. The amygdaloid sites from which stimulation suppressed predatory attack were localized to the medial nucleus (Fig. 1). The distribution within the amygdala is consistent with previous observations in our laboratory and elsewhere that the medial and basomedial aspects of amygdala generally suppress predatory attack, while more lateral regions, which include the central and lateral nuclei, facilitate it (Egger and Flynn, 1963; Block et al., 1980; Siegel and Brutus, 1990).

Medial amygdaloid modulation in the absence of ethanol

The effects of paired trials of single and dual stimulation conducted over a period of 200 min in the absence of drug administration are shown in Fig. 2. This observation indicates the magnitude of medial amygdaloid suppression as well as its relative stability over time. As indicated in this figure, medial amygdaloid stimulation suppressed predatory attack by 48% in the 5–25 min test period; suppression dropped slightly to 37% during the 60–80 min test period, and returned to 45% at the 180–200 min test period. The suppressive effects of medial amygdaloid stimulation upon predatory attack did not change significantly over time ($F = 0.50, df = 3,18, P = 0.79$). This level of response suppression thus served as a baseline for assessing how ethanol and/or bicuculline administration could alter medial amygdaloid suppression of predatory attack.

Effects of ethanol administration

Ethanol administration (in doses of 0.5 and 1.0 g/kg, i.p.) resulted in an overall significant dose- ($F = 48.7, df = 3.9, P < 0.001$) and time- ($F = 44.92, df = 3.9, P < 0.001$) dependent enhancement of the suppressive effects of medial amygdaloid stimulation upon predatory attack behaviour elicited from the lateral hypothalamus. Each of the two highest doses (1.0 and 0.5 g/kg) significantly enhanced the suppressive effects of
Fig. 2. Response suppression of predatory attack behaviour following dual stimulation of the medial amygdala and lateral hypothalamus relative to single stimulation of the lateral hypothalamus alone.

Response suppression remained relatively constant over a 200 min period in the absence of ethanol or bicuculline administration. During this time period, the extent of response suppression varied from 48 to 37% (n = 5). The numbers 5, 60, 120 and 180 min are used to represent the beginning of each of the 20 min blocks of time when dual stimulation procedures were employed for this and for the subsequent Figures. Each bar represents the mean ± SEM for this and the other subsequent graphs.

medial amygdaloid stimulation ($F = 39.44$, df = 3,18, $P < 0.001$; and $F = 21.93$, df = 3,18, $P < 0.001$, respectively). As shown in Fig. 3, for both of these doses, peak effects occurred at 60–80 min post-injection ($P < 0.05$, least significant difference test). Moreover, at 60 min post-injection, a dose-dependent effect of ethanol upon medial amygdaloid suppression of predatory attack was highly significant ($F = 84.86$, df = 3,18, $P < 0.001$) for each of the two higher doses employed ($P < 0.05$, least significant difference test). Administration of the lowest dose of ethanol (0.01 g/kg) had no effect upon suppression of attack ($F = 2.45$, df = 3,18, $P = 0.09$, n.s.)

Effects of bicuculline

The next phase of the study sought to identify the dose of bicuculline that, when microinjected into the lateral hypothalamus, could block the suppressive effects of medial amygdaloid stimulation upon attack behaviour elicited from the lateral hypothalamus. As shown in Fig. 4, microinjection of bicuculline into the lateral hypothalamus resulted in a significant dose- ($F = 34.01$, df = 2.6, $P < 0.001$) and time- ($F = 56.71$, df = 3.9, $P < 0.001$) dependent decrease in medial amygdaloid suppression of attack. The highest dose of bicuculline (i.e. 0.15 nmol) produced a 93% blockade of medial amygdaloid suppression at 5–25 min post-injection ($F = 68.76$, df = 3.9, $P < 0.001$). Highly significant reductions in medial amygdaloid suppression were also observed following infusion of 0.075 nmol of bicuculline ($F = 31.82$, df = 3.9, $P < 0.001$) in which the maximal effects were again observed 5 min post-injection. However, administration of the lowest dose of bicuculline (0.015 nmol) or 0.9% saline alone failed to modify the suppressive effects of medial amygdaloid stimulation ($F = 0.66$, df = 3.9, $P = 0.66$, n.s.; and $F = 2.74$, df = 3.9, $P = 0.10$, n.s., respectively). The presence of differential effects of dose level over time was
indicated by the significant interaction term of the overall ANOVA ($F = 30.52$, df = 6,18, $P < 0.001$).

**Interaction of ethanol and bicuculline: dual stimulation of the lateral hypothalamus and medial amygdala**

This experiment revealed how bicuculline microinjections into the lateral hypothalamus affected ethanol-induced enhancement of medial amygdaloid suppression of attack elicited from the hypothalamus (Fig. 5). Here, 0.15 nmol of bicuculline was microinjected into the lateral hypothalamus 60 min after administration of 1.0 g/kg of ethanol. Note that this dose of ethanol, by itself, produced its maximal enhancing effects upon medial amygdaloid suppression of predatory attack at 60 min post-injection. In the absence of bicuculline, 1.0 g/kg of ethanol potentiated the suppressive effects of medial amygdaloid stimulation by >50%. Following bicuculline administration, the effects of ethanol on medial amygdaloid suppression were almost entirely blocked (except at the 5 min post-injection time test) as medial amygdaloid modulation approached baseline levels. Specifically, mean levels of medial amygdaloid suppression following bicuculline infusion were significantly lower than the levels of suppression observed in the absence of bicuculline delivery ($P < 0.05$, least significant difference test). These results indicated highly significant dose- ($F = 107.02$, df = 1,12, $P < 0.001$) and time- ($F = 61.95$, df = 3,12, $P < 0.001$) dependent changes in medial amygdaloid suppression of predatory attack behaviour. Moreover, bicuculline infusion altered ethanol potentiation of medial amygdaloid suppression of predatory attack behaviour as revealed by a significant dose x time interaction ($F = 191.25$, df = 3,12, $P < 0.001$).
Interaction of ethanol and bicuculline: single stimulation of the lateral hypothalamus alone

In this experiment, we further sought to determine whether bicuculline administration could alter the suppressive effects of ethanol upon predatory attack elicited by single stimulation of the lateral hypothalamus. As shown in Fig. 6, microinjections of bicuculline produced a highly significant blockade of the suppressive effects of ethanol at both 5 and 60 min post-ethanol administration (F = 29.35, df = 1.6, P = 0.002). Administration of bicuculline alone had little effect upon predatory attack at both 5 and 60 min post-injection (P > 0.25). This finding further supports the view that the suppressive effects of ethanol are mediated through a GABAergic mechanism within the lateral hypothalamus.

DISCUSSION

The results of this study point to the important role of GABA_A receptors in the lateral hypothalamus in ethanol-mediated suppression of predatory attack behaviour. This conclusion is based upon the findings that: (1) the suppressive effects of medial amygdaloid stimulation upon predatory attack behaviour were potentiated by ethanol administration; (2) these suppressive effects (of medial amygdaloid stimulation) were blocked by bicuculline administration into the lateral hypothalamus; (3) ethanol's enhancement of these suppressive effects of the medial amygdala were blocked following local microinjections of the GABA_A receptor antagonist bicuculline, into the lateral hypothalamic attack site; (4) ethanol suppression
of predatory attack elicited by single stimulation of the lateral hypothalamus was significantly attenuated following bicuculline administration into the lateral hypothalamus. Thus, it is suggested that ethanol’s suppressive effects upon predatory attack may be accounted for in part by its actions on the pathway from the medial to lateral hypothalamus, including the GABA_A receptor mechanism within the lateral hypothalamus which receives synaptic contacts from medial hypothalamic neurons (Han et al., 1996b). In this respect, the present study provides new evidence identifying a suppressive function of ethanol upon a form of aggressive behaviour, predatory attack, and further establishes a likely neural circuit over which a component of this suppression is mediated. It should be noted, however, that the GABA_A receptor mechanism represents one way by which ethanol can suppress predatory attack behaviour. It is possible that ethanol suppression of predatory attack behaviour is also mediated through other structures, such as the basal amygdala (Egger and Flynn, 1963; Block et al., 1980; Shaikh et al., 1994) and septal area (Siegel and Skog, 1970; Brutus et al., 1984), which are known to suppress this form of aggressive behaviour. The target projection of the basal amygdala is the PAG, whose functions are partially mediated by NMDA receptors (Shaikh et al., 1994), and that of the septal area is the hypothalamus (Krayniak et al., 1980; Brutus et al., 1984), whose receptor mechanism remains
In view of the fact that ethanol has complex and non-specific properties and that it was administered peripherally, the question may be raised whether in fact the present data could have been the result of ethanol’s actions on neurons and receptors situated elsewhere in the brain. Several lines of evidence, however, argue against this possibility. Firstly, if the suppressive effects of ethanol were indeed non-specific, then it would be predicted that ethanol would have the same suppressive effects upon other forms of aggressive behaviour. However, it was recently observed that ethanol administration actually powerfully facilitated the occurrence of defensive rage behaviour elicited from the medial hypothalamus of the cat (Schubert et al., 1996a).

Secondly, the dual stimulation paradigm employed in the present study was specifically designed to identify the degree of specificity of ethanol upon the modulatory properties of the circuit arising from the medial amygdala. For example, if the effects of ethanol were indeed ‘non-specific’, then the pattern of results obtained would have been different from those observed in the present study. In such a case, ethanol administration would have caused an elevation of the threshold for predatory attack observed on trials of both single and dual stimulation, but would not have affected the magnitude of response suppression observed following dual stimulation involving the medial amygdala. This of course was not the case.

Thirdly, the observations that ethanol enhancement of (1) medial amygdaloid suppression of predatory attack, and (2) the suppressive effects upon this response elicited by single stimulation of the lateral hypothalamus were blocked by local microinjections of bicuculline into the lateral hypothalamus, provide further support for the specificity hypothesis. If other pathways were involved in mediating ethanol’s potentiating
effects upon response suppression, then the blocking effects of bicuculline within the lateral hypothalamus would likely have been significantly reduced.

As indicated above, our laboratory provided evidence that two forms of aggressive behaviour in the cat (predatory attack behaviour and defensive rage) are differentially affected by ethanol administration (Schubert et al., 1996a). In that study, it was proposed that these differential effects could be understood in terms of ethanol excitation of medial hypothalamic neurons. Such a hypothesis is logically possible. The recent discovery of several key pathways of the medial hypothalamus provides the structural basis for this view. Specifically, it has been shown that the expression of defensive rage is mediated through a descending pathway from the medial hypothalamus to the midbrain PAG (Lu et al., 1996b). In addition, some medial hypothalamic neurons contain short axons that project to the lateral hypothalamus. These neurons are inhibitory and their functions are mediated through GABA_A receptors in the lateral hypothalamus (Han et al., 1996b). Thus, such neurons could provide the substrate by which activation of the medial hypothalamus facilitates defensive rage (associated with the medial hypothalamic and its descending projection to the PAG), but inhibits predatory attack associated with the lateral hypothalamus. The observation that the medial amygdala, which projects directly to the medial hypothalamus, facilitates medial hypothalamically elicited defensive rage behaviour (Stoddard-Apter and MacDonnell, 1980; Shaikh et al., 1993), while producing in turn inhibition of predatory attack behaviour (Han et al., 1996a), can also be understood in terms of this circuit.

On the basis of the above discussion, the effects of ethanol's potentiation of neurons in the medial hypothalamus would be twofold: (1) facilitation of defensive rage by virtue of excitation of the descending pathway to the midbrain PAG, which provides the substrate for the expression of this response; and (2) suppression of predatory attack behaviour by virtue of an inhibitory projection from the medial to lateral hypothalamus. It should be noted that bicuculline's blockade of both the suppressive effects of ethanol upon predatory attack elicited by single stimulation of the lateral hypothalamus as well as upon ethanol's potentiation of the suppressive effects of medial amygdaloid stimulation can be understood in terms of the present hypothesis, namely by ethanol's initial potentiation of medial hypothalamic neurons, which in turn, suppresses the lateral hypothalamus via a GABAergic neuron. As indicated above, we have demonstrated that this circuit is also utilized in medial amygdaloid suppression of predatory attack by virtue of direct projections of this nucleus to the medial hypothalamus.

The question of the specific mechanism by which ethanol alters GABAergic transmission, causing an enhancement of medial amygdaloid suppression of attack, may also be raised in the present study. One suggestion is that ethanol has a potententiating effect upon an element of the inhibitory GABAergic projection from the medial to the lateral hypothalamus. Such a view is consistent with recent observations, suggesting that ethanol acts upon GABA receptors (Suzdak et al., 1986, 1988; Allan and Harris, 1987; Mehta and Ticku, 1988; Ticku, 1990, 1991; Hodge and Aiken, 1996). According to this view, ethanol may enhance GABAergic transmission by augmenting chloride flux (Suzdak et al., 1988; Ticku, 1991) through the GABA_A receptor complex, possibly by its actions upon the \(\alpha_1\beta_1\gamma_2L\) subunits (Criswell et al., 1993; Whitten et al., 1996). Other possible receptor locations include the benzodiazepine binding site, a depressant recognition site and a picrotoxin site, all of which affect chloride channels (Lister and Linnoila, 1991). Previously, it was shown that the effect of these actions could serve to modulate single-cell responses (Givens and Breese, 1990) and motor activity (Frye and Breese, 1982), as well as accounting for the reinforcing, anxiolytic (Liljequist and Engel, 1984; Koob et al., 1989), and discriminative (Hodge and Aiken, 1996) properties of ethanol. Therefore, it is possible that the enhancing effects of ethanol upon medial amygdaloid suppression of predatory attack behaviour are mediated through a similar mechanism in which ethanol acts upon GABA_A receptors within the lateral hypothalamus.

Two other possible mechanisms should also be suggested. One notion is that ethanol has a direct excitatory effect upon cell bodies within the medial hypothalamus. On the basis of our understanding of the circuitry described above (i.e. activation of the medial hypothalamus would
facilitate the mechanism for defensive rage but suppress that for predatory attack), this hypothesis is logically possible. However, the likely subcellular process by which such a mechanism occurs is presently not understood. The question may also be raised whether the effects observed in the present study could also be due to ethanol's possible inhibitory actions upon voltage-gated N or L calcium channels which, in turn, impact upon neurotransmitter release (Little, 1991). The problem with this view, however, is that if the effects of ethanol upon these calcium channels are inhibitory, then it would be difficult to explain how such a mechanism could account for the potentiating effects of ethanol upon medial hypothalamically elicited defensive rage behaviour (Schubert et al., 1996a).

In summary, the present study provided evidence that the suppressive effects of ethanol upon predatory attack are linked to a pathway from the medial to lateral hypothalamus whose functions are mediated by GABA_A receptors. Although it was suggested that ethanol's effects may be due to its direct actions upon GABA_A receptors, conclusions concerning the specific mechanism have yet to be clearly elucidated.

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