

Neurotensin Levels in Specific Brain Regions and Hypnotic Sensitivity to Ethanol and Pentobarbital as a Function of Time after Haloperidol Administration in Selectively Bred Rat Lines

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ABSTRACT

Evidence indicates that sensitivity to ethanol is a good predictor of the development of alcoholism. Thus, identification of neuronal processes that regulate ethanol sensitivity has been the subject of much recent research. The present studies were designed to further test the hypothesis that neurotensinergic processes mediate, in part, hypnotic sensitivity to ethanol. Single doses of haloperidol were administered to lines of rats [selectively bred for high and low sensitivity (HAS and LAS, respectively) to hypnotic effects of ethanol] to produce increases in neurotensin (NT) levels in brain regions. At 20 h after administration, haloperidol produced dose-dependent increases in NT immunoreactivity levels in nucleus accumbens (NA) and caudate putamen (CP) in both HAS and LAS lines.

Levels of NT in NA and CP returned to control values at 48 h after 4 mg/kg haloperidol. These studies used two measures of hypnotic sensitivity to ethanol: duration of loss of righting reflex (sleep time) and blood ethanol concentration at regain of righting reflex (BECRR). At 20 h, but not 48 h, after haloperidol treatment, both HAS and LAS rats displayed increases in ethanol-induced sleep time with concomitant decreases in BECRR. Pentobarbital-induced sleep time was not increased 20 h after administration of 4 mg/kg haloperidol; however, hypnotic sensitivity to both pentobarbital and ethanol was increased by acute (30-min) pretreatment with 1 mg/kg. These results suggest that NT levels in NA, acting via NT receptors, enhance hypnotic sensitivity to ethanol, but not pentobarbital.

Convincing evidence has been obtained supporting a neuromodulator role for neurotensin (NT) in the CNS. NT immunoreactivity (NT-ir) is widely distributed in mammalian brain (Cooper et al., 1981; Emson et al., 1982) with highest levels in the hypothalamus (HYP), ventral midbrain (VMB), and nucleus accumbens (NA). NT-ir is often colocalized with dopamine (Jennes et al., 1982; Bean et al., 1989). Electrical stimulation of the median forebrain bundle, *in vivo*, causes corelease of NT-ir and dopamine in the medial prefrontal cortex (MPFC) (Bean et al., 1989) and release of the peptide from brain slices is Ca²⁺-dependent (Iversen et al., 1978). Other microdialysis studies have demonstrated that NT enhances the release of GABA and acetylcholine in rat striatum (O'Connor et al., 1992; Tanganelli et al., 1994).

Pharmacological studies have established that NT modulates central dopaminergic and cholinergic functions. Neurotensin, administered directly into the cerebral ventricles

(*i.c.v.*) or into the NA elicits potent neuroleptic-like effects similar to those produced by classical antipsychotic drugs, *e.g.*, the dopamine antagonist haloperidol (Nemeroff, 1980). Administered into the MPFC, NT blocks the ability of dopamine to inhibit firing by pyramidal neurons (Shi and Bunney, 1992), some of which project to the NA (Carr et al., 1999). Neurotensin, injected *i.c.v.* or into the ventral striatum (including NA), blocks locomotor activation produced by cocaine and amphetamine (Kalivas et al., 1982). Recent studies have shown that NT injected into rat forebrain induces bursting activity of cholinergic basal forebrain neurons and an associated increase in θ -cortical activity together with an increase in paradoxical sleep (Cape et al., 2000). These results are consistent with the presence of high-affinity NT receptors (NTS1) on cholinergic forebrain cell bodies that project to the cerebral cortex (Szigethy and Beaudet, 1987).

The gene for preproneurotensin/neuromedin N (preproNT) has been cloned from rat and human (Dobner et al., 1987; Kislauskis et al., 1988; Bean et al., 1992). Levels of mRNA do

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ABBREVIATIONS: NT, neurotensin; CNS, central nervous system; NT-ir, neurotensin immunoreactivity; HYP, hypothalamus; VMB, ventral midbrain; NA, nucleus accumbens; MPFC, medial prefrontal cortex; GABA, γ -aminobutyric acid; NTS1, high-affinity NT receptors; preproNT, preproneurotensin/neuromedin N; CP, caudate putamen; BECRR, blood ethanol concentration at regain of righting reflex; SS, insensitivity; LS, sensitivity; HAS and LAS, lines of rats selectively bred for high and low hypnotic sensitivity to ethanol, respectively; ANOVA, analysis of variance; MFC, medial frontal cortex.

not always correspond with levels of NT, e.g., a mismatch was shown in CA1 of hippocampus and subiculum (Alexander et al., 1989). Dobner et al. (1992) reported a cooperative regulation of *preproNT* gene expression by transcription factors, including Fos, cAMP response element-binding protein, and glucocorticoids. Drugs that alter dopaminergic function, e.g., haloperidol, produce increases in *c-fos* and *preproNT* mRNA (Merchant et al., 1991; Merchant and Dorsa, 1993). These findings provide a basis for the results of Govoni et al. (1980), who observed increases in NT-ir levels 16 h after a single dose of 2 mg/kg haloperidol. Subsequently, others confirmed and extended these findings, showing that a range of acute and chronic doses of haloperidol given to rats increases NT-ir in the NA, CP, and ventral tegmental area (Radke et al., 1989; Levant and Nemeroff, 1992).

Experiments have implicated NT and NTS1 receptors in the regulation of hypnotic sensitivity to ethanol, i.e., ethanol-induced sleep time and blood ethanol concentration at regaining righting reflex (BECRR) in mice and rats. Widdowson (1987) demonstrated that i.c.v. injections of NT increased ethanol-induced sleep time in rats, and Luttinger et al. (1981) reported that i.c.v. administration of NT increased ethanol-induced sleep time in C57BL mice. Erwin et al. (1987) found that NT, injected i.c.v., dose dependently decreased blood ethanol concentration required to produce loss of righting reflex in SS/Ibg, but not LS/Ibg, mice; these mice were selectively bred for insensitivity (SS) and sensitivity (LS) to ethanol-induced sleep time. The ability of NT to increase hypnotic sensitivity to ethanol in SS mice was specific, in that pentobarbital-induced sleep time was not increased by central administration of NT.

Using 24 LS \times SS recombinant inbred strains of mice, Erwin et al. (1997) found significant genetic correlations between measures of hypnotic sensitivity to ethanol and densities of NTS1 receptors in striatum (including both NA and CP). However, with this limited sample size, ethanol sensitivity did not significantly correlate with NT-ir levels in the NA. Recent results (V. G. Erwin, V. Gehle, K. Davidson, and R. A. Radcliffe, manuscript submitted for publication) with an F2 generation derived from inbred LS and SS mice confirmed the significant correlation between striatal NTS1 receptor density and ethanol-induced sleep time and BECRR. Likewise, V. G. Erwin, V. Gehle, K. Davidson, and R. A. Radcliffe (manuscript submitted for publication) observed significant phenotypic correlations between ethanol-induced sleep time and BECRR and striatal NTS1 receptor densities in an F2 generation of rats, derived from HAS and LAS lines. These studies have strengthened the hypothesis that central neurotensinergic processes mediate, in part, differences in ethanol sensitivity. To further test this hypothesis, experiments were conducted to determine the effects of haloperidol-induced increases in endogenous neurotensin levels on measures of hypnotic sensitivity to ethanol.

Materials and Methods

Experimental Animals. Replicate lines of HAS (HAS1, HAS2) and LAS (LAS1, LAS2) were used in these studies. The respective HAS and LAS lines were selectively bred for 18 generations for high and low hypnotic sensitivity to ethanol (Draski et al., 1992). Subsequently, the lines have been maintained by random matings within-line for 19 generations; the HAS and LAS animals used in these

experiments were from this 19th generation. Animals were housed in facilities with 12/12-h light (6 AM-6 PM)/dark (6 PM-6 AM) cycles and with constant temperature ($22 \pm 2^\circ\text{C}$) and humidity ($40 \pm 2\%$). All experiments were performed using procedures approved by the University of Colorado Health Science Animal Care and Use Committee.

Brain Dissections and Neurotensin Radioimmunoassays.

For determinations of NT-ir levels, rats were anesthetized in a CO_2 chamber and sacrificed by decapitation; brains were rapidly removed, quickly chilled in ice-cold 0.9% NaCl (saline, containing 1 mM 1,10-phenanthroline, to inhibit NT degradation), and dissected on ice-cold aluminum-alloy blocks. Brain regions were quickly dissected according to the anatomical guidelines of Paxinos and Watson (1986) as previously described (Erwin et al., 1996). Tissue weights were recorded after dissection and each region was separately homogenized in 10 to 20 volumes of 0.01 N HCl extraction solution, heated in boiling water for 5 min, and centrifuged at 100,000g for 30 min. The resulting supernatant was transferred to fresh tubes, lyophilized, and stored at -80°C before assay. Standard double antibody radioimmunoassay procedures, routinely used in this laboratory, were used to measure levels of NT-ir (Erwin et al., 1997). The NT antiserum was obtained from Dr. Marvin Brown (University of California, San Diego, San Diego, CA) and has high specificity for NT; the antiserum recognizes the carboxyl-terminal portion of NT. Thus, it is possible that the antibody recognizes neuromedin N, but with lower sensitivity (M. Brown, personal communication).

Displacement curves were obtained by plotting the ratio of ^{125}I -NT (PerkinElmer Life Science Products, Boston, MA) tracer bound in the presence (B) and in the absence (B_0) of unlabeled NT (Sigma-Aldrich, St. Louis, MO) standards against the \log_{10} NT amount. Antiserum dilutions yielding approximately 30 to 40% binding of ^{125}I -NT tracer (15–20,000 cpm) in the absence of NT standards were used. Concentrations of NT-ir in tissue extracts were calculated by regression analysis of standard curves. Aliquots of the reconstituted extracts were assayed in triplicate with at least two dilutions. Extraction efficiency, determined by addition of NT_{1-13} to homogenates, was approximately 75 to 80%. Assay sensitivity was 5 to 100 pg of NT with an IC_{50} of 20 pg and an intra-assay coefficient of variation of approximately 5%.

Measurement of Hypnotic Sensitivity. Hypnotic sensitivity was measured, as previously described (Erwin et al., 1996), by both sleep time (duration of loss of righting reflex) and BECRR after 2.45- and 3.6-g/kg i.p. doses of ethanol in HAS and LAS, respectively. Differential doses of ethanol were required because ethanol sensitivity of the HAS lines had diverged from the LAS lines. Haloperidol HCl (Tocris Cookson, Ballwin, MO) was administered i.p. as described in the figure and table legends. Duration of pentobarbital-induced loss of righting reflex was measured after a 35-mg/kg dose. These studies were performed in a constant temperature (22°C) animal room. We routinely perform these tests and have repeatedly shown that blood and brain concentrations are equivalent (Erwin et al., 1987) after the initial rapid distribution phase, e.g., 4 to 5 min after injection i.p. Operationally, the criterion for righting is the animal being able to change from the supine to prone position three times in 30 s. At regaining righting a 25- μl blood sample is taken from the retro-orbital sinus followed by enzymatic-spectrophotometric assay for ethanol (Lundquist, 1959).

It is important to note that performing behavioral tests and brain dissections between 8:00 AM and 11:00 AM controlled for any potential circadian rhythms in ethanol sensitivity and in NT measures.

Statistical Analyses. All one- and two-way analyses of variance (ANOVA), post hoc tests (Dunnett's *t* tests), and bivariate product moment (Pearson's) correlations were performed using SPSS 9.0 (SPSS, Inc., Chicago, IL). The *F* statistics and significance values are shown in the tables and figures.

Results

In previous studies we compared the BECRR and brain NT-ir values in HAS and LAS rats from generation 17 of selective breeding (Erwin et al., 1996). The results presented in Table 1 from subsequent generations (see *Materials and Methods*) show that replicate lines of LAS have maintained a low ethanol sensitivity (BECRR = 350–439 mg/dl ethanol) and replicate lines of HAS have become even more sensitive to the hypnotic effect of ethanol (BECRR = 180–204 mg/dl ethanol). Males and females from HAS and LAS lines did not differ significantly in sensitivity to ethanol. As previously reported (Erwin et al., 1996) NT-ir levels were highest in HYP followed by NA > VMB > CP > MFC and there were no significant line differences in NT-ir levels, except for values in NA that were significantly higher in HAS1 than in LAS1. The previous report indicated small sex differences in NT levels in various brain regions for both HAS and LAS lines; however, in the present study those results were not replicated (Table 1), thus in the present studies data from males and females were combined.

As shown in Table 2, 20 h after single doses of haloperidol, levels of NT-ir were significantly increased in NA and CP from the replicate lines of HAS and LAS rats. These results are consistent with those of Govoni et al. (1980), Radke et al. (1989), and Levant and Nemeroff (1992), who found haloperidol-induced increases in NT levels in discrete regions of rat brain. The haloperidol-induced increases in NT-ir were dose-dependent with no change at 1 mg/kg, slight increases at 2 mg/kg, and up to 4-fold increases with a dose of 4 mg/kg. At these doses, haloperidol produced no significant increases in NT-ir levels in HYP, VMB, or MFC, except for an increase in MFC from LAS2 at 4 mg/kg.

To determine whether haloperidol pretreatment alters hypnotic sensitivity to ethanol, rats were injected with single doses of haloperidol 20 h before ethanol administration (Table 2). As noted in Figs. 1 and 2, ANOVA showed significant

dose effects of haloperidol on ethanol-induced sleep time and BECRR. Replicate HAS and LAS lines, treated with haloperidol doses of 2 and 4 mg/kg, but not 1 mg/kg (LAS2), were significantly more sensitive to the hypnotic effects of ethanol than saline control animals. After 4 mg/kg haloperidol, HAS1 and HAS2 sleep time values were increased 53 and 55% (97 and 86 min), respectively, and in LAS1 and LAS2 lines sleep time scores were increased 164 and 100% (59 and 64 min), respectively (Fig. 1). The corresponding reductions in BECRR were 69 and 42 mg/dl in HAS1 and HAS2 rats and 46 and 50 mg/dl in LAS1 and LAS2 animals, respectively. The reductions in BECRR are consistent with increases in sleep time in that these rat lines metabolize ethanol at approximately 50 mg/dl/h (Dahchour et al., 2000).

Phenotypic correlations (Pearson's product moment) between measures of hypnotic sensitivity to ethanol and NT-ir levels in NA and CP were calculated for HAS (HAS1 and HAS2 combined) and for LAS (LAS1 and LAS2 combined) lines (Table 3). The data show significant correlations between NT-ir levels in NA, but not in CP, and sleep time and BECRR in HAS animals and between NT-ir levels in NA, but not in CP, and BECRR in LAS lines. As expected, NT-ir levels in NA and CP were highly correlated. The significant correlation between ethanol sensitivity and NT levels in NA, but not CP, might indicate that basal forebrain (including NA) NT mediates increases in ethanol sensitivity.

In Table 4 values are presented for measures of hypnotic sensitivity to ethanol and NT-ir levels in NA and CP in HAS and LAS lines 48 h after a single dose of 4 mg/kg haloperidol. Results show that haloperidol-treated rats were similar to control animals in each of these measures, indicating a return to control levels of NT-ir and hypnotic sensitivity.

To determine whether the effect of haloperidol pretreatment on ethanol sensitivity was drug-specific, pentobarbital-induced sleep time (duration of loss of righting reflex) was measured in HAS and LAS rats 20 h after saline or 4 mg/kg haloperidol (Fig. 3). Surprisingly, the results showed that control LAS1 rats had significantly shorter sleep time values than control HAS1. However, control HAS2 and LAS2 sleep time values were virtually identical, indicating that the HAS1 versus LAS1 difference was not a result of selective breeding, but probably a result of fortuitous gene fixation during selective breeding for ethanol sensitivity. It is of interest that the sleep time for the haloperidol-treated animals was not significantly different from respective controls for any line, indicating that the effect of haloperidol pretreatment on hypnotic sensitivity to ethanol was drug-specific.

Because haloperidol has CNS-depressant properties, it was possible that some of the effect of haloperidol (20-h pretreatment; Figs. 1 and 2) on hypnotic sensitivity to ethanol was due to the presence of residual haloperidol in brain. Determining the effects of acute haloperidol (1/mg/kg) administration on ethanol as well as pentobarbital sensitivity tested this possibility. As shown in Table 5 haloperidol administered 30 min before both ethanol and pentobarbital increased sensitivity to both of these agents. These findings suggest that if residual haloperidol had been present in brain 20 h after its administration, increased sensitivity to pentobarbital as well as ethanol would be expected. Thus, the most parsimonious explanation for the increase in ethanol sensitivity 20 h after haloperidol administration is an altered CNS

TABLE 1
Comparisons of blood ethanol concentrations at regain of righting reflex and brain neurotensin levels in HAS and LAS rats

As noted under *Materials and Methods*, because HAS and LAS rats were selectively bred to differ in the duration of ethanol-induced loss of righting reflex (sleep time), differential doses of ethanol (2.45 and 3.6 g/kg, respectively) were administered to these rat lines. Differential hypnotic sensitivity was determined by measuring the BECRR. Neurotensin-ir was determined as described under *Materials and Methods*. In preliminary studies, it was shown that NT-ir levels in brain regions from both naive and ethanol-treated rats were not significantly different. Therefore, in all subsequent experiments rats were sacrificed immediately after the regain of righting reflex. Values are expressed as mean \pm S.E.M. ($n = 4-6$ animals/line/sex). There were no significant effects of sex in ethanol sensitivity or NT-ir levels within individual lines. Therefore, data for males and females were combined in subsequent experiments. There was a significant overall line effect for BECRR ($F_{3,41} = 87, p < 0.10^{-5}$), but not for NT-ir levels in NA or CP. NT-ir levels in NA from HAS1 were significantly higher than from LAS1 ($F_{1,21} = 4.0, p = 0.05$).

Rat Line	Sex	BECRR	Neurotensin-Immunoreactivity Levels	
			Nucleus Accumbens	Caudate Putamen
		mg/dl	ng/g of brain	
HAS1	M	193 \pm 10	10.8 \pm 1.6	4.4 \pm 1.2
HAS1	F	204 \pm 12	12.0 \pm 1.7	6.2 \pm 2.0
HAS2	M	180 \pm 14	5.2 \pm 2.1	2.7 \pm 2.1
HAS2	F	203 \pm 10	10.6 \pm 2.9	6.1 \pm 1.8
LAS1	M	439 \pm 19	6.3 \pm 2.0	2.9 \pm 1.5
LAS1	F	400 \pm 17	6.9 \pm 1.5	5.2 \pm 1.6
LAS2	M	383 \pm 17	6.1 \pm 1.6	3.1 \pm 0.9
LAS2	F	347 \pm 20	10.0 \pm 2.1	3.0 \pm 0.7

TABLE 2

Neurotensin levels in brain regions of HAS and LAS rats 20 h after haloperidol administration

Rat brains were dissected and neurotensin immunoreactivity was determined in NA, CP, MFC, VMB, and HYP 20 h after administration of haloperidol i.p. in the doses indicated. Values represent the means \pm S.E.M. ($n = 8-12$ /line/dose) with approximately equal numbers of males and females in each line and dose.

Rat Line	Haloperidol Dose	Neurotensin-Immunoreactivity				
		NA	CP	MFC	VMB	HYP
	mg/kg			ng/g of tissue		
HAS1	0	11.2 \pm 2.8	6.2 \pm 2.1	3.8 \pm 1.2	8.6 \pm 1.2	29.5 \pm 2.1
HAS1	2	14.3 \pm 3.0	6.0 \pm 0.7	1.7 \pm 0.3	7.8 \pm 0.7	24.0 \pm 3.5
HAS1	4	21.2 \pm 4.6 ^a	18.8 \pm 4.7 ^a	6.6 \pm 2.0	9.8 \pm 2.1	30.2 \pm 4.6
LAS1	0	6.5 \pm 1.0	3.6 \pm 0.6	2.0 \pm 0.3	9.0 \pm 0.9	27.9 \pm 2.8
LAS1	1	9.5 \pm 1.7	5.0 \pm 0.5	1.1 \pm 0.2	6.9 \pm 1.6	27.6 \pm 2.2
LAS1	2	12.3 \pm 1.1	10.2 \pm 1.2	2.5 \pm 0.9	11.8 \pm 1.3	23.4 \pm 3.1
LAS1	4	13.2 \pm 2.2 ^b	11.7 \pm 1.0 ^b	2.5 \pm 0.8	9.4 \pm 1.1	30.8 \pm 3.3
HAS2	0	7.8 \pm 1.5	4.4 \pm 1.0	2.7 \pm 0.7	7.3 \pm 0.7	27.6 \pm 3.2
HAS2	2	10.5 \pm 1.7	7.8 \pm 1.1	2.1 \pm 0.4	7.4 \pm 0.9	24.4 \pm 4.0
HAS2	4	12.9 \pm 1.8 ^c	8.9 \pm 1.4 ^c	2.5 \pm 0.6	9.3 \pm 1.3	30.1 \pm 2.2
LAS2	0	8.3 \pm 2.9	3.0 \pm 0.6	2.2 \pm 0.5	10.0 \pm 3.0	33.0 \pm 3.7
LAS2	2	9.7 \pm 1.3	7.1 \pm 0.8	2.0 \pm 0.2	6.7 \pm 0.6	27.8 \pm 3.6
LAS2	4	26.8 \pm 6.0 ^d	20.8 \pm 5.1 ^d	7.6 \pm 2.1 ^d	12.5 \pm 3.3	35.2 \pm 4.3

Significant dose effects were observed as follows: ^a HAS1, $F_{2,29} = 6.2$ and 4.2 , $p < 0.05$, for NA and CP, respectively; ^b LAS1, $F_{3,52} = 3.5$ and 8.0 , $p < 0.02$ and 10^{-3} , for NA and CP, respectively; ^c HAS2, $F_{2,35} = 2.9$ and 3.3 , $p < 0.05$, for NA and CP, respectively; ^d LAS2, $F_{2,37} = 5.0$, 5.3 , and 3.8 , $p < 0.05$, for NA, CP, and MFC, respectively. Values at haloperidol doses of 2 and 4 mg/kg (except for HAS1) differed significantly, $p < 0.05$, from 0 dose controls by Dunnett's t tests.

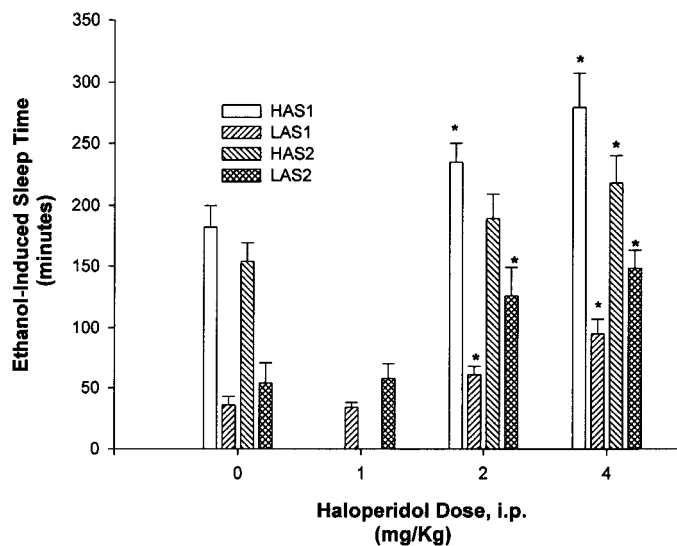


Fig. 1. Dose-dependent effects of haloperidol on ethanol-induced sleep time in HAS and LAS rats. Haloperidol was administered i.p. 20 h before determination of the ethanol-induced duration of loss of righting reflex (sleep time) as described under *Materials and Methods*. As expected control (0 haloperidol dose) HAS and LAS lines differed markedly in sleep time. Significant haloperidol dose effects were observed as follows: HAS1, $F_{2,33} = 5.8$, $p < 0.005$; HAS2, $F_{2,33} = 6.1$, $p < 0.005$; LAS1, $F_{2,52} = 9.0$, $p < 10^{-3}$; and LAS2, $F_{2,36} = 3.5$, $p < 0.05$. Sleep time values are expressed as mean \pm S.E.M. with degrees of freedom indicated in the F statistics listed above. Haloperidol (4 mg/kg)-treated rats displayed a 60- to 90-min increase in ethanol-induced sleep time. Values indicated by an asterisk (*) differed significantly, $p < 0.05$, from respective 0 haloperidol controls by Dunnett's t tests.

sensitivity produced by increases in NT-ir levels, or other neuronal processes, in specific brain regions.

Discussion

In the present study we have confirmed that single doses of haloperidol (1–4 mg/kg) increase NT-ir levels in NA and CP and to a lesser extent in MPFC 20 h, but not at 48 h, after drug administration. It is well documented that these doses of haloperidol increase *c-fos* and preproNT mRNA levels within a few hours in discrete rat brain regions (Merchant et

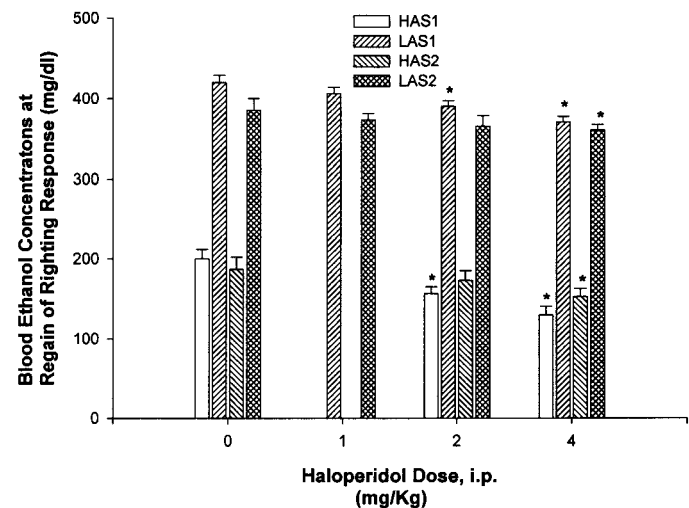


Fig. 2. Dose-dependent effects of haloperidol on blood ethanol concentrations at regain of righting reflex in HAS and LAS rats. The experiment was the same as described in Fig. 1. At the regain of righting reflex after ethanol doses of 2.45 and 3.6 g/kg in HAS and LAS rats, respectively, retro-orbital blood samples were immediately taken and blood ethanol concentrations (BECRR) were determined as described under *Materials and Methods*. Significant haloperidol dose effects were observed as follows: HAS1, $F_{2,33} = 14.3$, $p < 10^{-4}$; HAS2, $F_{2,33} = 4.7$, $p = 0.01$; LAS1, $F_{2,52} = 10.1$, $p < 10^{-4}$; and LAS2, $F_{2,36} = 6.4$, $p < 0.005$. BECRR values were decreased by 45 to 70 mg/dl in the 4 mg/kg haloperidol-treated rats. Values indicated by an asterisk (*) differed significantly, $p < 0.05$, from respective 0 haloperidol controls by Dunnett's t tests.

al., 1991; Merchant and Dorsa, 1993). A likely sequence of haloperidol effects is an increase in *c-Fos* followed by preproNT expression leading to increased NT-ir synthesis. Because *Fos*, a transcription factor, regulates expression of many genes, it is possible that an increase in *Fos* levels results in enhanced levels of neuronal peptides (proteins) other than NT. However, the present results provide support for the hypothesis that NT levels in discrete forebrain regions regulate hypnotic sensitivity to ethanol. For example, the data show significant correlations between NT levels in NA and hypnotic sensitivity to ethanol, suggesting that the increase in NT levels mediated the increase in ethanol sensitivity. Moreover, the time course for effects on ethanol sen-

TABLE 3

Correlations among measures of hypnotic sensitivity to ethanol and neurotensin levels in nucleus accumbens and caudate putamen from HAS and LAS rats

For calculation of phenotypic correlations (Pearson's) between measures, values for HAS1 and HAS2 and for LAS1 and LAS2 animals from Table 2 were combined. Correlations for HAS ($n = 64$) and LAS ($n = 89$) animals are shown above and below the diagonal, respectively.

Measure	Sleep Time	BECRR	NA NT-ir Levels	CP NT-ir Levels	
Sleep time		-0.72*	0.49	0.11	HAS
BECRR	-0.64*		-0.31*	-0.01	
NA NT-ir	0.12	-0.44*		0.65*	
CP NT-ir	-0.03	0.20	0.80		LAS

*Significant correlations, $p < 0.01$.

TABLE 4

Measures of hypnotic sensitivity to ethanol and neurotensin levels in brain regions of HAS and LAS rats 48 h after haloperidol administration

HAS and LAS rats were tested for hypnotic sensitivity to ethanol (sleep time and BECRR) and neurotensin levels in the NA and caudate putamen 48 h after a 4-mg/kg dose of haloperidol or saline. Mean values for sleep time, BECRR, or NT levels in NA or CP did not differ between control (0 dose) and haloperidol-treated animals.

Rat Line	Haloperidol Dose	Sleep Time	BECRR	Neurotensin Immunoreactivity	
				CP	NA
		<i>min</i>	<i>mg/dl</i>	<i>ng/g of tissue</i>	
HAS1	0	233 ± 16	178 ± 14	4.0 ± 1.0	8.0 ± 2.1
HAS1	4	201 ± 15	177 ± 11	6.1 ± 1.2	9.8 ± 1.7
HAS2	0	182 ± 13	201 ± 11	3.8 ± 0.6	6.4 ± 1.5
HAS2	4	164 ± 9	212 ± 10	4.1 ± 1.0	8.4 ± 0.8
LAS1	0	30 ± 6	452 ± 15	3.6 ± 0.8	3.9 ± 0.6
LAS1	4	32 ± 5	460 ± 16	5.0 ± 0.9	5.1 ± 1.1
LAS2	0	38 ± 9	429 ± 13	4.2 ± 0.6	6.2 ± 1.3
LAS2	4	41 ± 10	421 ± 17	3.2 ± 0.7	5.9 ± 0.6

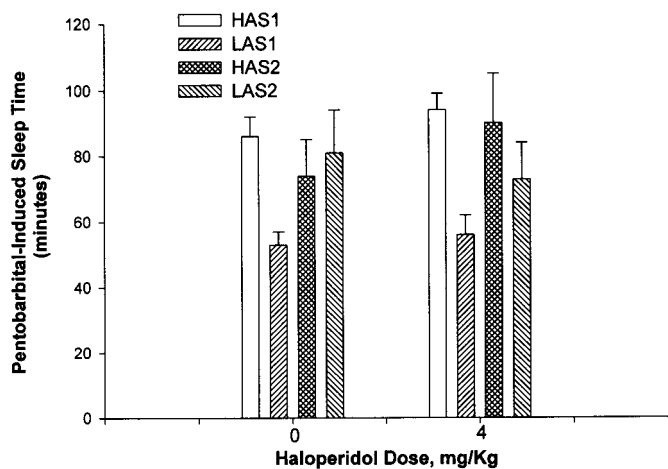


Fig. 3. Absence of effects of haloperidol pretreatment on pentobarbital-induced sleep time in HAS and LAS rats. Haloperidol (4 mg/kg) or saline were administered i.p. 20 h before determination of pentobarbital-induced duration of loss of righting reflex (sleep time) as described under *Materials and Methods*. Sleep time values are expressed as mean ± S.E.M. ($n = 6-8$ /line/dose with equal numbers of males and females). ANOVA showed no significant main effects for haloperidol dose in any of the rat lines.

sitivity coincide with the time course for changes in striatal NT levels, i.e., the effect of haloperidol on hypnotic sensitivity to ethanol was seen at 20 h, but not 48 h, after haloperidol administration.

Because it is expected that the presence of haloperidol, a CNS depressant, in the brain would increase ethanol sensitivity (Table 5), it was important to rule out the possibility that residual haloperidol was responsible for enhanced ethanol sensitivity in rats treated with haloperidol 20 h before ethanol administration. This was shown in part by the finding that rats treated with 4 mg/kg haloperidol were not more sensitive to pentobarbital at 20 h after haloperidol, even though 1 mg/kg haloperidol increased pentobarbital, as well

as ethanol, sensitivity when administered only 30 min before haloperidol (Table 5). Moreover, studies of the time course for haloperidol levels in blood and brain after i.p. injections show that levels peak in approximately 1 h followed by a rapid decrease to undetectable levels at 6 h after 1 mg/kg and to about 2% (0.2 nmol/g of brain) of the peak level at 12 h after 5 mg/kg (Campbell et al., 1980). In those studies, haloperidol levels in brain were higher than in blood; however, blood and brain levels decreased in parallel. A pharmacokinetic study in rats showed a linear decrease in blood haloperidol concentrations with a half-life of 1.5 h (Cheng and Paalzw, 1992). These results provide strong support for the assumption that 20 h after 1- to 4-mg/kg doses, haloperidol was cleared from brain and that the increases in hypnotic sensitivity to ethanol were not due to interaction between ethanol and haloperidol.

The results in the present study are consistent with previous observations that hypnotic sensitivity to ethanol is enhanced by i.c.v. administration of NT in both mice and rats (Frye et al., 1981; Luttinger et al., 1981; Erwin et al., 1987; Widdowson, 1987). Erwin et al. (1987) showed that the effects of centrally administered NT on hypnotic sensitivity to ethanol were neuropeptide-specific, and ethanol-specific, i.e., NT i.c.v. did not alter hypnotic sensitivity to pentobarbital. The latter finding is of interest in that in the present study, increases in endogenous NT (20 h after haloperidol administration) did not alter sensitivity to pentobarbital, indicating a drug specificity for endogenous NT effects on sensitivity to ethanol.

In previous studies we showed that HAS and LAS lines of rats had similar levels of NT in discrete brain regions, including the NA and CP (Erwin et al., 1996). These findings were confirmed in the present study. Because NT levels are regulated, in part, by polygenetic influences in LS × SS recombinant inbred strains of mice (Erwin et al., 1997), it is likely that NT levels in NA and CP are genetically regulated

TABLE 5

Effects of acute haloperidol administration on hypnotic sensitivity to ethanol and pentobarbital

Haloperidol (1 mg/kg i.p.) or saline were administered 30 min before administration of ethanol (2.45 or 3.6 g/kg in HAS2 and LAS2, respectively) or pentobarbital (35 mg/kg). Hypnotic sensitivity was measured by ethanol-induced duration of loss of righting reflex (sleep time), BECRR, and by pentobarbital-induced sleep time. Values represent the mean \pm S.E.M. ($n = 6$ –8/line/dose, males only).

Rat Line	Haloperidol Dose	Ethanol-Induced Sleep Time	BECRR	Pentobarbital-Induced Sleep Time
	mg/kg	min	mg/dl	min
HAS2	0	161 \pm 26	194 \pm 19	121 \pm 31
HAS2	1	259 \pm 9 ^a	105 \pm 11 ^a	228 \pm 29 ^b
LAS2	0	49 \pm 12	397 \pm 8	90 \pm 31
LAS2	1	191 \pm 20 ^a	99 \pm 17 ^a	200 \pm 33 ^b

Acute haloperidol administration produced significant increases in hypnotic sensitivity to ethanol or pentobarbital as follows: ^a Haloperidol-treated animals differ from controls (0 dose) for ethanol-induced sleep time and BECRR for HAS2, $F_{1,16} = 16.0$ and 18.1 , $p < 0.001$, respectively; and for LAS2, $F_{1,16} = 29.0$ and 21.1 , $p < 0.001$, respectively. ^b Values for pentobarbital-induced sleep time differ from controls (0 haloperidol dose) for HAS2 and LAS2, $F_{1,12} = 5.7$ and 5.5 , $p < 0.05$, respectively.

in rats. Consequently, one might expect differences in NT levels in HAS and LAS rats, selectively bred for differences in hypnotic sensitivity to ethanol. One explanation is that all alleles that contribute to ethanol sensitivity, particularly those with small effect size, may not be completely segregated after only 18 generations of selection.

The hypothesis that neurotensinergic processes mediate, in part, hypnotic sensitivity to ethanol is strengthened by recent observations that NTS1 densities are correlated with hypnotic sensitivity to ethanol in an F2 population derived from HAS and LAS rats (V. G. Erwin, V. Gehle, K. Davidson, and R. A. Radcliffe, manuscript submitted for publication). Thus, in considering the activity of neurotensinergic mechanisms that may mediate hypnotic sensitivity, both NT levels and NTS1 densities in discrete brain regions must be considered. Several possible mechanisms could account for the ability of NT to enhance ethanol sensitivity. It is known that NT, injected into the cerebral ventricles (i.c.v.) or ventral striatum (including NA) blocks locomotor activation produced by cocaine and amphetamine (Kalivas et al., 1982). The mechanism for NT-induced locomotor inhibition is unknown. However, Tanganelli et al. (1994) reported that NT facilitates GABA release associated with a reduction of dopamine release in the NA, and it is well known that ethanol potentiates GABA-induced inhibition of neuron firing (Mihec and Harris, 1996). Recent studies have shown that NT injected into rat forebrain induces bursting activity of cholinergic basal forebrain neurons and an associated increase in θ -cortical activity together with an increase in paradoxical sleep (Cape et al., 2000). These results are consistent with the presence of NTS1 on cholinergic forebrain cell bodies that project to the cerebral cortex (Szigethy and Beaudet, 1987). Ehlers et al. (1999) demonstrated a reduction in overall electroencephalographic spectral power induced by i.c.v. NT injections in P and NP rats. The P and NP rats, selectively bred for differences in voluntary ethanol consumption, have been shown to differ in hypnotic sensitivity to ethanol (Lumeng et al., 1982) and in NT levels in amygdala, frontal cortex, and caudate (Ehlers et al., 1999).

The present results of specific pharmacological interactions between ethanol sensitivity and endogenous NT levels in the NA and CP, coupled with significant correlations between ethanol sensitivity and NT levels in the NA strengthen the hypothesis that central neurotensinergic processes mediate, in part, differences in hypnotic sensitivity to ethanol.

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