strongly (not shown). In the small and large intestines, absorptive cells, goblet cells, and Paneth cells were devoid of detectable aldolase C, but neuroendocrine cells were positive. Lymphocytes and neutrophils were stained (fig. 5). Control sections were uniformly negative (fig. 6). Formalin-fixed, paraffin-embedded sections of pancreas showed a remarkable reduction in the stainability of aldolase C.

Discussion. There are several proteins specific to or associated with neurons and neuroendocrine cells; γ-enolase (neuron-specific enolase; NSE)[6-10], chromogranin[13,14], creatine kinase BB (CK-B)[15], and α subunit of guanine nucleotide-binding protein (Goα)[16]. All of these are acidic proteins. Since their levels in the sera of patients with neuroendocrine tumors increase, the proteins could well be clinically applicable as serum markers for the diagnosis of neuroendocrine tumors. In addition, because such proteins are immunohistochemically detectable in neuroendocrine cells and the tumor cells derived from these cells, the immunohistochemical detection of these antigens is employed for differential diagnosis in the field of diagnostic pathology.

Aldolase C is also known to be distributed mainly in the central nervous system[1-5]. Sato et al. suggested that studies of the aldolase isozyme might be useful in the diagnosis of brain tumors by comparing the isozyme patterns of various brain tumors with those of normal brain[17]. Willson et al. suggested that the aldolase C level of cerebro-spinal fluid might reflect damage within the central nervous system[18]. Kumanishi et al. reported that aldolase C staining was prominent in astrocytes and Purkinje cells, although faint staining was also occasionally observed in some other neurons[4]; its localization had not been shown in the peripheral organs.

In this report, we examined the localization of the aldolase C in the peripheral neuroendocrine tissues, and demonstrated that it is localized in neuroendocrine cells of various types. The γ-enolase, another glycolytic enzyme, is a well-known marker for neuroendocrine cells[6-10]. It was initially considered to be neuron-specific, but subsequently was found to be present in non-nervous cells or tissues[10]. When the tissue distribution of aldolase C was compared with that of γ-enolase, the stainability of aldolase C in neuroendocrine cells was similar to that of γ-enolase. Aldolase C, however, was not neuron-specific like γ-enolase, since positive staining was also observed in non-neuroendocrine cells such as some surface epithelia of stomach, lymphocytes and neutrophils, with the use of PLP-fixed, cryostat sections. The distribution of aldolase C in non-neuroendocrine tissues is quite different from that of γ-enolase[10]. These findings suggest that the immunohistochemical application of aldolase C in diagnostic pathology should be undertaken with care, in order to avoid the same confusion that is seen with the use of γ-enolase[19]. In addition, the antigenicity of aldolase C in formalin-fixed, paraffin-embedded sections was found to be remarkably reduced as compared to that in PLP-fixed, cryostat sections; this might also be a disadvantage in applying aldolase C in clinical pathology.

Acknowledgments. We thank Y. Yamamura, MD and T. Kito, MD, Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, for their valuable suggestions and discussions during the course of these studies. Mrs C. Yamada of the laboratory provided excellent technical assistance.

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0014-4754/88/090749-03$1.50 + 0.20/0

Narcotic antagonism of seizures induced by a dopamine-derived tetrahydroisoquinoline alkaloid

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Received 2 March 1987; accepted 30 May 1988

Summary. This paper describes experiments designed to evaluate whether the narcotic antagonist naloxone significantly interferes with seizures induced by tetrahydroisoquinolines (TIQs). In these experiments we found that naloxone significantly reduced seizure scores induced by intra-cranially infusing mice with 50 μg of the dopamine-derived tetrahydroisoquinoline (TIQ) alkaloid, 6,7-dihydroxy TIQ. These findings support an opioid involvement in the actions of TIQs and may lead to further understanding of opioid-mediated novel excitatory receptors.
Key words. Tetrahydroisoquinoline; naloxone; CNS excitation; opioids; dopamine.

After scrutinizing the literature, our laboratory undertook the present study to determine whether the narcotic antagonist naloxone can alter ethanol-induced intoxicating actions in a dose-dependent fashion and whether naloxone can similarly antagonize seizures produced by simple TIQ compounds.

To date most animal research has shown that the addictive agents ethanol and opiates share common biochemical mechanisms of action[1-5]. Despite some reports to the contrary[6-8], most researchers in this field generally agree that some of ethanol's actions occur by activating opiate receptors[9-14]. Studies by Davis and Walsh[15] and others[16] postulate that benzyl TIQs share common properties with opiates. Ross's group has supported this argument[17-20], while Hamilton and co-workers have found that opiate antagonists (naloxone or naltrexone) have attenuated the opiate-

like effects of these TIQs [21,22]. Furthermore, TIQs have been shown to alter binding of met-enkephalin at its endogenous receptor site [23,24].

In the past our laboratory has shown that naloxone significantly attenuates ethanol-induced withdrawal seizures [25]. In other research, we have speculated that ethanol-induced withdrawal seizure activity is mediated by TIQs formed following ethanol administration and that the TIQs cause these seizures by interacting with opioid receptors [26]. Sjoquist et al. [27,28] and others [29] have done human research indicating that higher levels of TIQs are formed in chronic alcoholics than in non-alcoholics.

Therefore, we suspect that TIQs may be responsible for hangover-effects, particularly tremors. If research can find a method to block these seizures with narcotic antagonists, then narcotic antagonism may become useful as a clinical adjunct in treating alcoholism.

To systematically evaluate the above hypothesis, we designed the first experiment to test the dose-dependent anti-ethanol behavioral action of naloxone. We utilized a previously developed method to rapidly evaluate acute ethanol intoxication in mice [30]. Briefly, this evaluation method entailed the use of an apparatus, consisting of a metal bar (6.4 mm diameter) fixed between two metal plates and suspended 27 cm above an electrified grid. The day before assessing ethanol intoxication, we trained ICR Swiss mice to remain on the bar every 30 min for a total of 5 h, resulting in 33 trials per mouse. We used the same voltage, usually between 15–35 V, for both plates and grid for each mouse every time it was placed on the bar. We used a 10-s holding-time trial for the training sessions and scored ethanol intoxication according to the method of Blum et al. [30].

The animals received an i.p. injection of ethanol 4.5–12% v/v dissolved in saline and doses of ethanol ranged from 0.9 to 2.4 g/kg b.wt. Each animal received 0.0263 ml ethanol solution per g body weight. Ethanol concentration was adjusted for each dose: for example, animals receiving 1.5 g/kg were given 7.5% ethanol v/v, while animals receiving 2.4 g/kg received an injection of 12% ethanol v/v. In this experiment we determined that the ED_{50} of ethanol to induce behavioral intoxication was 1.05 g/kg. Thereafter in the experiment we utilized this dose. We dissolved the narcotic antagonist naloxone in 0.5% saline and gave it in doses ranging from 4 to 10 mg/kg. We injected naloxone or saline i.p. 30 min prior 1.05 g/kg. Five min after the ethanol injection, we placed each animal on the bar for three trials. We derived their intoxication scores by subtracting the number of seconds they remained on the bar from the number ten. Thus, short bar-holding times resulted in high intoxication scores.

Figure 1 illustrates the protective effect of naloxone on ethanol-induced behavioral intoxication. In this experiment, 4 mg/kg of naloxone reduced the ethanol-induced motor impairment by 27%, whereas 6 mg/kg of naloxone resulted in a higher protective action of 49% of the saline-ethanol effect, which was significant at the $p < 0.05$ level. However, unlike the 4 mg/kg and 6 mg/kg dose of naloxone, mice receiving 10 mg/kg of naloxone showed overt signs of CNS stimulation. In fact, administering 10 mg/kg of naloxone yielded an intoxication score 144% higher than that of the saline-ethanol group; mice receiving this high dose of naloxone actually jumped over the bar within seconds of being placed in proper position.

This property observed for naloxone may now help explain Goldstein and Judson's early findings [6]. They reported that naloxone at 55 mg/kg (a dose within the seizure-range) given to mice physically dependent upon ethanol did not modify the course of the alcohol-withdrawal syndrome. We believe that this dose of naloxone and even smaller doses could have contributed to the withdrawal seizure scorings, thereby masking any inhibitory effect of the narcotic antagonist on the alcohol withdrawal syndrome. Finally, we have observed that administration of naloxone at 55 mg/kg to the Texas Inbred strain of mice resulted in seizures followed by death in 100% of animals so tested.

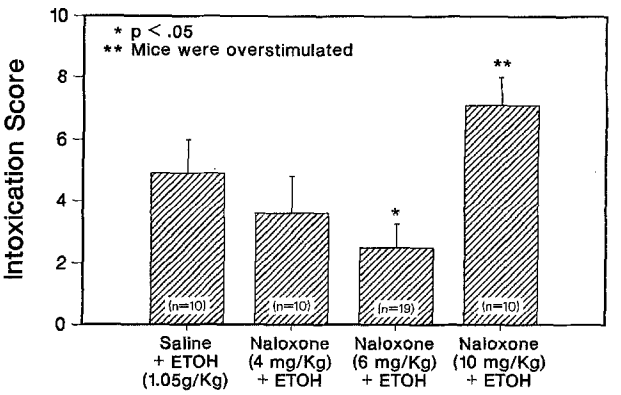

Figure 1. Illustrates the biphasic effects of naloxone on ethanol-induced intoxication in ICR Swiss mice. Saline or naloxone at 4, 6, and 10 mg/kg was injected i.p. 30 min prior to the intoxication score (IS) of ethanol at 1.05 g/kg. N = number of mice utilized in this study. The Student's t-test was employed to evaluate the significance. Whereas 4 and 6 mg/kg reduced ethanol intoxication, only at the 6 mg/kg dose was it significant. The 10 mg/kg dose produced overstimulation of 144%. The study used a total of 49 mice.

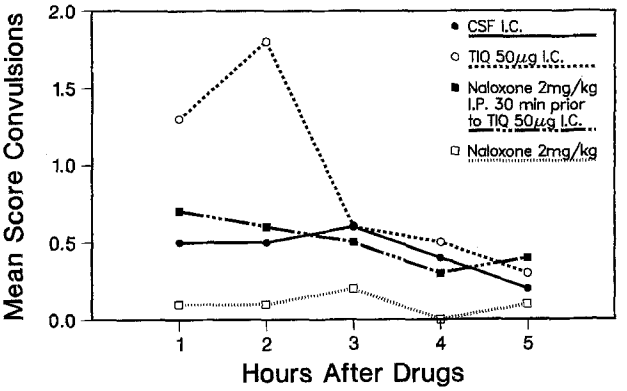

Figure 2. Illustrates the effect of intracerebral injections (see text) on the convulsive response in mice for 5 h following the administration of the drugs. Control *Ss* (10 µl) received i.c. injections of artificial cerebral spinal fluid, in an equivalent volume, as the TIQ injected *Ss* (10 µl). At least 10 mice were used in each group.

Since neuroamine-derived alkaloids intensify alcohol-withdrawal seizures [26] and produce hyperexcitability in mice, we designed a second experiment to determine whether an opiate antagonist could affect seizures produced by a simple TIQ intracerebral injection.

First, we randomly assigned male Swiss-Webster mice (19–25 g, Texas Inbred Co.) to four groups of ten animals each. Then we administered either artificial cerebral spinal fluid (CSF) (control), or naloxone alone (2 mg/kg i.p.), or 6,7-dihydroxy-TIQ alone (50 µg/animal i.c.), or 6,7-dihydroxy-TIQ (50 µg/animal i.c.) 30 min after naloxone (2 mg/kg i.p.). Every hour for the 5 h after drug administration, we determined drug-induced seizure scores. We performed intra-cerebral injections (µl) using a 27-gauge stainless-steel needle according to Haley and McCormick's method [31]. We dissolved 6,7-dihydroxy-TIQ in CSF, and drug amounts refer to weights of the bases. We used a seizure-scoring method previously described [32].

Preliminary experiments indicated that the mean seizure score for untreated mice (0.26 ± 0.06) did not differ significantly from that of mice receiving 10 µl of CSF (0.32 ± 0.07) or 2 mg/kg naloxone. Intracerebral infusion of 50 µg of 6,7-dihydroxy-TIQ produced a peak seizure score of 1.8 ± 0.2 2 h after administration (fig. 2). This effect had terminated by the 3rd h. Utilizing the Student's t-test, we determined that pretreatment of the mice with naloxone significantly reduced ($p < 0.001$) the 6,7-dihydroxy-TIQ-induced seizures (fig. 2). In these experiments, we utilized no ethanol, so that the study would focus on CNS excitability in naive animals. Anti-TIQ stimulatory response of naloxone peaked at 2 mg/kg, so we chose this dose in the final presentation of these results.

We hypothesize that alcohol withdrawal seizures may be partially mediated by activation of opioid excitatory receptors. Reports from our laboratory [33, 34] and others [3, 5, 36] indicate that both opiates and ethanol equally subserve withdrawal seizures by providing common agonist receptor interactions. Since TIQ is a by-product of alcohol and is slowly metabolized, it may induce seizure activity by directly activating an unidentifiable special opioid receptor, as suggested by Collins [37]. This opioid receptor may be the endogenous site for α-endorphin, a psychostimulant [38], which may be increased during ethanol administration as others observed with brain peptides [39]. Therefore, the question we raise is whether the TIQs induce hyperexcitability indirectly by displacing α-endorphins or directly by activating their endogenous excitatory receptor site.

Finally, our finding that naloxone blocks the 6,7-dihydroxy-TIQ-induced hyperexcitability warrants further exploration of novel excitatory receptors that are mediated by opioids, such as the PCP-sigma site [40].

This research was supported by the Air Force Office of Scientific Research Grant and by Matrix Technologies, Inc., Houston, Texas. The author acknowledges Boris Tabakoff for supplying the formula of the CSF used in this experiment. While the experiments were being conducted, K. Blum was a Career Teacher in Drug Addiction sponsored by NIDA Grant No. 1-TO1-DA00290-01. The author is grateful to Dr Gerald Cohen for supplying the 6,7-dihydroxytetrahydroisoquinoline for these studies. Editorial assistance was provided by Eileen Knoff, Office of Educational Resources, University of Texas Health Science Center at San Antonio.

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