creased A-H interval significantly more than diltiazem alone (table). In the presence of propranolol, diltiazem also increased AVERP (table). These results can be accounted for by a potentiation of diltiazem effects on AV node by propranolol. As control values were measured after propranolol administration in the diltiazem-propranolol series, a significant difference between the 2 series does not mean a simple addition of effects but rather a potentiation. Propranolol increases A-H interval and AVERP[11] by antagonizing the effects of released catecholamines. Calcium current is increased by catecholamines[12], which have an effect on AV node opposite to that of calcium blockers[13,14]. As the slight but significant fall in blood pressure due to diltiazem induces not only a reflex depression of vagal tone[9] but a stimulation of sympathetic activity, the slow channel blocker action on AV node is normally restricted. It is restricted less, or not at all, in the presence of propranolol. A potentiation might result in complete AV block with higher doses of one or both drugs. Such a conduction defect was not observed here in therapeutic doses[9,11]. It nevertheless warns of the possible occurence of conduction troubles with a diltiazem-beta blocker combination in clinical practice.

1 Acknowledgments. Financial support from the U.E.R. Biologie Humaine is gratefully acknowledged.
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## Whole brain methionine-enkephalin of ethanol-avoiding and ethanol-preferring C57BL mice

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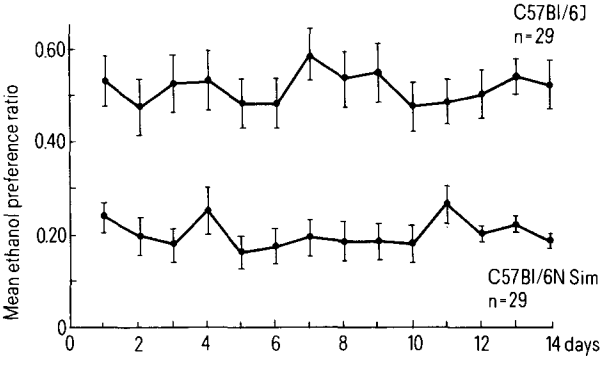
*Summary.* These experiments systematically investigated ethanol preference in both the C57Bl/6N and C57Bl/6J mice utilizing three-choice 2-bottle preference test. In addition, these sublines were evaluated for whole brain methionine-enkephalin levels, which were significantly lower in C57Bl/6J mice (alcohol preferring) compared to C57Bl/6N mice (alcohol non-preferring). This finding supports the involvement of the peptidyl opiates in ethanol seeking behavior.

It is well known that mice of the C57BL family of inbred strains are widely used in psychogenetic research dealing with alcohol because of their generally high preference for 10% ethanol solutions in 2-bottle, ethanol vs water preference tests[1-5]. Previously, certain C57BL subline mice were found to display clear ethanol avoidance in a 2-bottle preference test[5]. During routine experimentation in our laboratory, we similarly found that C57Bl/6N mice obtained from Simonsen Laboratories avoided ethanol in the same test situation in which C57Bl/6J mice from the Jackson Laboratories displayed a 'C57BL-typical' high preference for ethanol. As proposed by Poley[5], the difference in alcohol preference might be due to differences in rearing conditions between Simonsen and Jackson colonies or genetic differences between the sublines. In terms of genetic determinants of ethanol preference, our laboratory proposed the 'Psychogenetic theory of drug seeking behavior'. The basis of the theory resides in the possibility that drug or alcohol seeking behavior is a function of endogenous peptidyl opiate levels[6,7]. Therefore, ethanol-preferring mice should possess a genetic deficiency of peptidyl opiates, whereas ethanol-avoiding mice should possess relatively higher levels of peptidyl opiates. Some evidence to support the involvement of the peptidyl opiate system in ethanol preference is derived from the findings that C57BL mice (alcohol preferring) show a genetic deficiency of enkephalin when compared to DBA (non-preferring) mice[8,9].

The experiments reported here were conducted to further characterize C57BL preference behavior with regard to ethanol. In the first experiment, we systematically investi-

Comparative whole brain methionine-enkephalin levels in sublines of C57 mice

| Subline | Methionine-enkephalin (pm/g brain) | N | Significance |
|---|---|---|---|
| C-57 Bl/6N Sim | 323.84 ± 13.58 | 10 | $p < 0.05$ |
| C-57 Bl/6J | 289.36 ± 14.27 | 10 | |



gated ethanol preference in both the C57Bl/6N and C57Bl/6J mice utilizing the 3-choice, 2-bottle preference test. In the 2nd experiment, animals of C57Bl/6N and C57Bl/6J sublines were evaluated for whole brain met-enk levels to see if differences in the sublines, if obtained, support or refute the 'psychogenetic theory' of ethanol-seeking behavior[6].

C57Bl/6N Simonsen and C57Bl/6J (8 weeks old) were obtained from Simonsen Laboratories (Gilroy, California) and Jackson Laboratories (Bar Harbor, Maine), respectively. Methionine-enkephalin radioimmunoassay kits were obtained from Immunonuclear Corporation (Stillwater, Mn 55082).

The mice were all on a 12L:12D cycle, housed 15/cage (1240 $cm^2$), and were acclimatized 7 days in our laboratory prior to use. Both C57Bl/6J and C57Bl/6N Sim were decapitated and their respective brains were removed and frozen on a block of ice. The whole brains were weighed and homogenized in 5.0 ml of a 0.1 N HCl or 1 M HAc solution. The homogenates were centrifuged 14,000 × g for 15 min in a J21B centrifuge.

The supernatant was removed and frozen at − 10 °C overnight and the next day was recentrifuged under the same conditions. 50 µl of the above solution were transferred to 5.0-ml tubes over ice and dried with a steady stream of nitrogen. The samples were redissolved in 750 µl of 0.01 M borate-0.1% BSA buffer (pH 8.4) and 200 µl of this was assayed for methionine enkephalin. Duplicate samples were run and a normalized %-bound standard curve was employed to determine levels which were corrected for efficiency.

In other groups, C57Bl/6N Sim and C57Bl/6J mice were placed on a 14-day preference routine using the 3-choice/2-bottle method of Myers and Holman[10]. Ethanol (10%)/tap water and tap water consumption were measured every day. The fluids were administered in top sealed inverted 12 $cm^3$ syringes with a standard laboratory right angle drinking spout. Throughout the procedure, animal weights were monitored and were found either to be constant or to increase slightly.

In the figure, C57Bl/6J show a much greater preference of ethanol (10%) than the C57Bl/6N Sim mice. The 14-day mean preference ratio of ethanol for the 29 C57Bl/6N Sim was $0.20 \pm 0.012$, which was significantly ($p < 0.001$) lower than the 29 C57Bl/6J which was $0.52 \pm 0.015$.

Absolute ethanol consumption in C57Bl/6J and C57Bl/6N was $6.367 \pm 0.0895$ (414 = N) and $2.77 \pm 0.140$ g/kg (316 = N), $p < 0.01$, respectively. Average fluid consumption in C57Bl/6J and C57Bl/6N was $4.23 \pm 0.053$ ml/day (404 = N) and $5.10 \pm 0.098$ ml/day, $p < 0.01$, respectively.

The table illustrates the met-enk whole brain levels in C57Bl/6N and C57Bl/6J mice. 10 C57Bl/6N mice had $323.84 \pm 13.58$ pm/g brain tissue, of met-enk; whereas, 10 C57Bl/6J had $289.36 \pm 14.27$ pm/g brain tissue. Thus, C57Bl/6J mice were found to possess significantly lower ($p < 0.05$) met-enk levels compared to C57Bl/6N mice.

These studies demonstrate that 1. C57Bl/6N (Simonsen) clearly avoid ethanol, whereas C57Bl/6J (Jackson) possess the 'C57BL typical' high ethanol preference; 2. C57Bl/6J mice possess significantly less whole brain met-enk levels compared to C57Bl/6N animals. These results support Poley's[5] suggestion of a genetic difference between C57BL sublines affecting alcohol preference and further suggests that the genetic difference may, in part, reside in the genotypic difference of whole brain met-enk levels.

Utilization of C57BL sublines with different preference for ethanol could be valuable genetic material for the investigation of the etiology of alcohol preference and for the study of the commonalities of alcohol and opiate addictions[11-13].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## Contraction of the large conductance coronary artery produced by acetylcholine in the mini pig


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*Summary.* In the mini pig, acetylcholine reduced the coronary blood flow and constricted the large coronary artery. These effects were abolished by atropine, but not by phentolamine, suggesting that cholinergic mechanisms may be involved in coronary artery vasoconstriction.


Coronary artery spasm has been established as a cause of Prinzmetal's variant angina both in patients with normal coronary arteries and in those with obstructive coronary artery lesions[1-4]. However, the mechanism by which the spasm of the coronary artery is initiated remains unknown. The multiple physiological and pharmacological stimuli reported to induce attacks suggest that underlying autonomic dysfunction may be a cause. The involvement of alpha-adrenergic mechanisms has been demonstrated[5]. Although it has been shown[6] that methacholine or pilocarpine could induce the attack and that atropine was effective in preventing this, the role played by cholinergic mechanisms has not been well defined, for cholinergic mechanisms were usually thought to be associated with vasodilation.

Very recently, Sakai[7] found in the isolated perfused heart preparation of the monkey and the mini pig a rise in the