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 ± 0.012, which was significantly ($p < 0.001$) lower than the 29 C57Bl/6J which was 0.52 ± 0.015.

Absolute ethanol consumption in C57Bl/6J and C57Bl/6N was 6.367 ± 0.0895 (414 = N) and 2.77 ± 0.140 g/kg (316 = N), $p < 0.01$, respectively. Average fluid consumption in C57Bl/6J and C57Bl/6N was 4.23 ± 0.053 ml/day (404 = N) and 5.10 ± 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 ± 13.58 pm/g brain tissue, of met-enk; whereas, 10 C57Bl/6J had 289.36 ± 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

perfusion pressure after an initial fall following administration of methacholine and bethanechol that could be abolished by atropine. However, acetylcholine produced a rise in the perfusion pressure only in the monkey and only at high doses.

In view of this inconsistency and of the possible importance of the cholinergic mechanisms in the genesis of coronary vasospasm, the effects of acetylcholine on the coronary circulation, particularly on the large conductance artery, were examined in the mini pig. The results show that acetylcholine always produces a constriction of the large coronary artery in this species, together with a decrease in coronary blood flow.

Materials and methods. Six mini pigs of both sexes weighing 22–26 kg were anesthetized with the i.v. administration of urethane (250 mg/kg) and chloralose (25 mg/kg) after premedication with ketamine hydrochloride (10 mg/kg i.m.). The trachea was intubated, and the animal was artificially ventilated with air. The rate and stroke volume of the respirator were adjusted to maintain arterial oxygen tension at about 100 mm Hg and pH at about 7.4. The chest was opened through left thoracotomy. The small portion of the anterior descending branch of the left coronary artery (LAD) was isolated by blunt dissection approximately 2 cm below the bifurcation of the left coronary artery, cannulated and perfused at a constant pressure with blood taken from the femoral artery with the aid of an electronic constant pressure device (Datagraph). Prior to cannulation the animal was heparinized (500 U/kg i.v.). Additional doses of 100 U/kg were given at 1-h intervals. An electromagnetic flowmeter probe (Statham SP 2201) placed between the cannula and the constant-pressure perfusion device measured the coronary blood flow, and a pressure transducer (Statham P-50) connected to a side arm of the perfusion system recorded the coronary perfusion pressure (Pa).

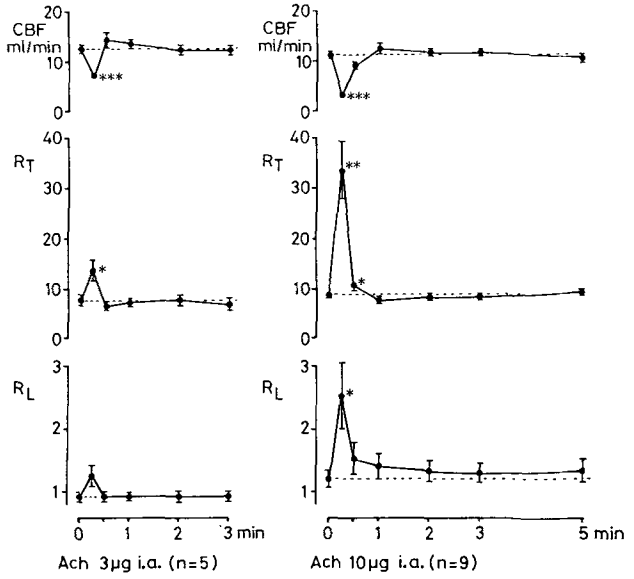
A small distal branch of the LAD was carefully exposed close to the apex of the heart, and a fine polyethylene cannula was inserted in a retrograde fashion into the vessel, to which a pressure transducer (Statham P-50) was connected in order to measure the peripheral coronary pressure (Pc). The pressure gradient (Pg = Pa − Pc) was also directly recorded using a Sanborn differential pressure transducer (Model 267B) interposed between the 2 cannulae. Also measured were the left ventricular pressure (LVP) with a catheter tip transducer (Miller P-350), its first derivative (dp/dt), and the aortic pressure (Statham P-50). These parameters were recorded on a Mingograf 800.

The heart rate was continuously monitored with a cardiotachometer triggered by the R wave of the ECG. The Pa, Pc, Pg, the coronary blood flow, heart rate and aortic pressure were displayed on a linearly recording thermostylus oscillograph (Mark V, Watanabe Sokki). The total resistance of the anterior descending branch (R_T) is calculated as: Pa/flow, and the resistance of the large conductance artery (R_L) as Pa-Pc/flow[8,9]. The drugs used were: urethane (Wako Chemicals), α-chloralose (Wako Chemicals), ketamine (Ketalar, Sankyo), acetylcholine chloride (Sankyo), atropine sulfate (Wako Chemicals) and phentolamine mesylate (Ciba-Geigy).

Results and discussion. The figure shows the effects of acetylcholine (3 and 10 μg) on the coronary blood flow, R_T and R_L. Injection of acetylcholine (3–10 μg) into the rubber tubing leading to the cannula of the anterior descending branch resulted in a dose-related decrease in coronary blood flow, indicating an increase in the total coronary resistance (R_T). A slight increase in the flow was observed following the decrease, but the changes were not significant. The resistance of the large coronary artery (R_L) was also increased dose-dependently. This is in contrast to the results of Sakai[7], who found only dilatation with doses similar to those used in the present study.

Although Sakai's data were not obtained with the constant pressure perfusion method but with the constant flow perfusion method, we were able to demonstrate a constriction (data not shown) even with the constant flow perfusion method. At present we have no explanation for this discrepancy. LVP, max dp/dt, aortic pressure and the heart rate were all slightly decreased, thus making a gross increase in extravascular resistance unlikely. Therefore, the increase in the R_L may be ascribed to the direct action of acetylcholine on the smooth muscle of the large coronary artery. The increase in the R_L and R_T were both abolished after treatment of the preparation with 1 mg of atropine. Phentolamine (1 mg) did not modify these effects of acetylcholine.

Thus, the observed increase in the R_L, as well as in the R_T, was due to the stimulation of muscarinic cholinergic receptors. Although the doses of acetylcholine used in the present study were rather high, the presence of very active cholinesterases in the blood and tissues still makes it reasonable that the observed constriction of the large conductance artery may be related to the vasospastic changes reported in man.



Effects of close i.a. injection of acetylcholine (Ach, 3 and 10 μg) on the coronary blood flow (ml/min), the total coronary resistance in arbitary units (R_T) and the resistance of the large coronary artery also in arbitary units (R_L) of the mini pig. Each point represents the mean ± SE. *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (t-test, paired data).

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