19 Withers, H.R., Hunter, N., Barkley, H.T., and Reid, B.O., Radiat. Res. *57* (1974) 88.
20 Dubravsky, N., Hunter, N., and Withers, H.R., Radiat. Res. *65* (1976) 481.
21 Potten, C.S., J. invest. Derm. *55* (1970) 410.
22 Baserga, R., Lisco, H., and Kisieleski, W., Proc. Soc. exp. Biol. Med. *110* (1962) 687.
23 Baserga, R., Lisco, H., and Kisieleski, W., Radiat. Res. *29* (1966) 583.
24 Potten, C.S., J. invest. Derm. *58* (1972) 180.
25 Dry, F.W., J. Genet. *16* (1926) 287.
26 Post, J., and Hoffman, J., Radiat. Res. *34* (1968) 570.
27 Post, J., and Hoffman, J., Radiat. Res. *45* (1971) 335.
28 Johnson, H.A., and Cronkite, E.P., Radiat. Res. *11* (1959) 825.
29 Olsson, L., Radiat. Res. *68* (1976) 258.
30 Bedford, J.S., Mitchell, J.B., Griggs, H.G., and Bender, M.A., Radiat. Res. *63* (1975) 531.
31 Grisham, J.W., Proc. Soc. exp. Biol. Med. *105* (1960) 555.
32 Cheng, H., and Leblond, C.P., Am. J. Anat. *141* (1974) 537.
33 Potten, C.S., in: Cell proliferation in the gastrointestinal tract, p.141. Ed. D.R. Appleton, J.P. Sunter and A.J. Watson. Pitman Medical, Tunbridge Wells 1980.

Interaction of diltiazem with propranolol on atrioventricular conduction and refractoriness in the dog[1]

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Summary. Propranolol pretreatment (0.1 mg · kg^{-1}) significantly increased the lengthening induced by diltiazem (0.15 mg · kg^{-1} + 0.01 mg · kg^{-1} · min^{-1}, 20 min) of the A-H interval of His bundle potential recordings in the dog. In the presence of propranolol, diltiazem also significantly increased the atrioventricular effective refractory period. The results suggest the possible occurrence of AV blocks as a result of the use of a diltiazem-beta blocker combination in clinical practice.

Calcium blockers are sometimes used in combination with beta-adrenergic blockers to treat severe and unstable angina pectoris. Such combinations have been proved to be dangerous with verapamil[3,4], but seem much safer with nifedipine[5]. In the present study, the effects of the new calcium blocker diltiazem were assessed in the dog in normal conditions and after beta-adrenergic blockade by propranolol.

Materials and methods. 12 mongrel dogs weighing from 18 to 22 kg were anesthetized with choralose (80 mg · kg^{-1}) and were given dextromoramide 0.1 mg · kg^{-1} i.v. 10 min before control measurements. Dextromoramide was used to restore vagal tone[6] which was reduced by chloralose anesthesia[7]. The atrioventricular effective refractory period (AVERP) was then longer than the atrial ERP and could thus be measured. The animals were intubated and ventilated by means of a respirator (Bird MK VIII), using a mixture of air and oxygen. His bundle potentials were recorded under right atrium pacing at a constant rate, according to the technique of Sherlag[8]. Atrial, AV node and ventricular ERPs were determined by the extrastimulus method (for details, see Lièvre et al.[9]). Mean blood pressure was continuously recorded through a catheter percutaneously introduced into the right femoral artery. The body temperature was maintained between 38 and 39 °C by means of external heating.

The study comparing diltiazem alone and a diltiazem-propranolol combination was made after randomization of the animals into 2 groups. In 6 animals, diltiazem 0.15 mg · kg^{-1} was injected i.v. just after control measurements and simultaneously infused at the rate of 0.01 mg · kg^{-1} · min^{-1} for 30 min. In 6 dogs, propranolol 0.1 mg · kg^{-1} was administered 10 min before control measurements, followed by diltiazem administration. Measurements were performed before (control) and 10, 20, 30 min after the beginning of diltiazem administration. In each series, comparisons of means with the control state were made by Student's t-test for paired values. Changes in parameters from control values were compared between the 2 series by Student's t-test for unpaired values after a comparison of variances had proved it possible.

Results and discussion. Diltiazem alone had the same effects on AV conduction as other slow channel inhibitors[9,10] and increased A-H interval (i.e. conduction time through AV node). AVERP was not significantly increased (table). Mean blood pressure decreased slightly but significantly from 98.3 ± 11.6 to 88.3 ± 13.8 mm Hg ($p < 0.05$). There were no significant changes in S-A and HV intervals, heart rate, atrial and ventricular ERPs. Propranolol lengthened the A-H interval. The A-H interval control value was therefore higher in the diltiazem-propranolol combination series. The difference between control values of AVERP was not significant. Diltiazem-propranolol combination in-

Effects of diltiazem (0.15 mg · kg^{-1} + 0.01 mg · kg^{-1} · min^{-1} for 30 min) and diltiazem-propranolol combination (diltiazem, same dose, propranolol 0.1 mg · kg^{-1}, 10 min before control measurements) on A-H interval of His bundle potential recordings and atrioventricular effective refractory period (AVERP)

		Control	10 min	20 min	30 min
A-H	Diltiazem (n = 6)	51.3 ± 4.3	63.6 ± 7.1	63.6 ± 3.4[a]	64.6 ± 5.5[a]
(ms)	Diltiazem + P (n = 6)	92.0 ± 9.8	118.3 ± 7.9[b]	115.0 ± 8.8[c,d]	116.0 ± 8.1[c]
AVERP	Diltiazem (n = 6)	233.2 ± 37.5	241.6 ± 32.8	246.6 ± 27.8	235.0 ± 17.4
(ms)	Diltiazem + P (n = 6)	278.3 ± 19.9	331.6 ± 27.0[a]	348.3 ± 25.4[a,d]	355.0 ± 26.1[b,d]

Mean values and SEM before (control) and 10, 20, 30 min after the beginning of diltiazem administration. n, Number of experiments. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$: significantly different from control value (paired t-test); [d] $p < 0.05$: comparison of changes in parameters from their control values between the 2 series.

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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3 Benaim, M.E., Br. med. J. *2* (1972) 169.
4 Denis, B., Pellet, J., Machecourt, J., and Martin-Noël, P., Nouv. Press Med. *23* (1977) 2075.
5 Fox, K., Jonathan, A., and Selwyn, A., Concours méd. *102* (1980) suppl. 21, 97.
6 Roquebert, J., Canellas, J., Dumartin, A., and Sabathie, M., Archs int. Pharmacodyn. Thér. *167* (1967) 297.
7 Arfors, K.E., Artuson, G., and Malmberg, P., Acta phsyiol. scand. *81* (1971) 47.
8 Sherlag, B.J., Helfant, R.H., and Damato, A.N., J. appl. Physiol. *25* (1968) 425.
9 Lièvre, M., Descotes, J., Brazier, J.L., Timour Chah, Q., and Faucon, G., Archs int. Pharmacodyn. Thér. *252* (1981) 272.
10 Kawai, C., Konishi, T., Matsuyama, H., and Okazaki, H., Circulation *63* (1981) 1035.
11 Seides, S.F., Josephson, M.E., Badsford, W.P., Weisfogel, G.M., Lau, S.H., and Damato, A.N., Am. Heart J. *88* (1974) 733.
12 Reuter, H., and Scholtz, H., J. Physiol., Lond. *264* (1977) 49.
13 Dhingra, R.C., Winslow, E., Pouget, J.M., Rahimtoola, S.H., and Rosen, K.M., Am. J. Cardiol. *32* (1973) 629.
14 Ollagnier, M., Lièvre, M., Descotes, J., Evreux, J.C., and Faucon, G., J. Pharmac., Paris *10* (1979) 173.

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