lated by the pineal gland, but that stimulating release of hypothalamic LHRH induced by severe lack of sex hormones after gonadectomy cannot be prevented by a stimulation of pineal function.

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Ethanol preference in rats with a prior history of acetaldehyde self-administration

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Summary. Peripherally self-injected acetaldehyde in interaction with environmental and nutritional variables significantly enhances alcohol drinking in rats and suggests an involvement of acetaldehyde in voluntary alcohol intake.
Key words. Rat; ethanol preference; acetaldehyde self-administration.

The importance of acetaldehyde in the regulation of voluntary ethanol intake in experimental animals is the subject of considerable debate[1-8]. Recently we reported that naive rats will self-inject i.v. significant amounts of acetaldehyde when placed at 80% reduced body weight (80% b.wt) on a 1-h daily FI 60-sec food delivery schedule over a period of 10 days[9]. This finding led us to consider the possibility that i.v. self-injection of acetaldehyde may affect an animal's consumption and preference for ethanol. To test this possibility rats were given the opportunity to self-infuse i.v. either acetaldehyde or saline before being presented with a choice of alcohol and water. In such situations the animals could choose ethanol for its pharmacological effects and/or as an alternative liquid or energy source, the amount of ethanol consumed being determined by a variety of regulatory mechanisms[7,9,10]. We report here an alteration of alcohol drinking in the rat by peripherally self-administered acetaldehyde. We suggest an involvement of acetaldehyde in the development of an animal's preference for alcohol.

Details of the procedure for inducing i.v. acetaldehyde self-administration have been reported elsewhere[9]. Two groups of 9 male Long-Evans hooded rats, surgically implanted with jugular catheters and reduced to 80% of their free feeding body weights were maintained on a 12:12 light/dark cycle (dark period beginning at 12.00 h) and allowed to self-inject a 1% v/v acetaldehyde solution (2.32 mg/kg/infusion) for a period of 20 days. Two similar groups were allowed to self-inject saline during this period.

In this procedure the rats were placed individually in operant chambers for 1 h/day over the 20 consecutive days, at the same time each day, with acetaldehyde or saline available i.v. through bar pressing. When an animal pressed the operant bar, the pump was activated for 5 sec and an infusion of fluid (0.07 ml) was delivered into the jugular vein. During the 5-sec infusion interval, additional presses did not reactivate the pump and were not recorded. All infusions during each 1-h test session were automatically monitored on cumulative recorders. Throughout the entire 20-day test period a FI 60-sec food delivery schedule was in operation with Noyes food pellets (45 mg) delivered non-contingently at the rate of 1 pellet per min. All rats were tested within 5 h after onset of the dark period and under red light conditions. At the end of 20 days plus a 2-day period without drug available for self-administration, 1 acetaldehyde and 1 saline group maintained at 80% b.wt were tested over 10 days for their preference for ethanol with tap water as an alternative fluid. During the 10-day period, the ethanol (95%) solutions offered to the animals were increased systematically in concentrations from 3 to 30% using a 3-bottle 2-choice technique[11]. The 2nd acetaldehyde and saline control groups were placed on free feeding conditions 2 days prior and during the 10-day ethanol preference sequence. A 2-way analysis of variance (groups × days, with repeated measures over the days factor) carried out on the self-injection data, showed significant main effects of drug treatments ($F(3,24) = 7.429$, $p < 0.01$) and days ($F(19,456) = 3.039$, $p < 0.001$) at a type 1 error rate of 0.05. Post hoc analysis with Newman-Keul's comparisons showed that animals in acetaldehyde (AcH) group 1 self-injected significantly more acetaldehyde ($p < 0.01$) than saline relative to animals in control groups 1 and 2. A significant difference ($p < 0.05$) was also found between animals in acetaldehyde group 2 and animals in both saline control groups. No significant differences between the two acetaldehyde groups or between the 2 saline control groups were shown. Animals with malfunctioning catheters were excluded from the analysis. Data showing the mean number of acetaldehyde and saline self-infusions by the animals over 20-day test period are presented in figure 1.

A 2-way analysis of variance (groups × days, with repeated measures over the days factor) performed on the ethanol

drinking data (g of ethanol per kg b.wt), yielded significant main effects for groups ($F(3,32) = 68.5$, $p < 0.001$) and days ($F(9,288) = 60.9$, $p < 0.001$). Newman-Keul's comparisons revealed that under both body weight conditions, animals allowed to self-administer acetaldehyde showed a significantly greater preference for alcohol over water compared with animals allowed to self-administer saline ($p < 0.01$). However, the proportion of alcohol in the mean daily fluid intake of animals at 80% b.wt who self-administered acetaldehyde was considerably greater than that observed with free feeding animals. Indeed, a similar pattern was observed in the animals allowed to self-administer saline. Figures 2a and 2b show respectively, the mean g of ethanol per kg b.wt consumed by each animal for each concentration of ethanol offered during food deprivation and free feeding conditions and mean proportion of ethanol to total fluid intake consumed by each group of rats for each concentration of ethanol offered during the 10-day preference sequence.

A significant correlation[12] was found between i.v. acetaldehyde self-administration and voluntary intake of ethanol (g/kg) for animals in acetaldehyde group 1 maintained at 80% b.wt ($r = 0.686$, $p < 0.05$) but not for animals in acetaldehyde group 2 placed on free-feeding conditions during the 10-day ethanol preference sequence ($r = 0.233$, $p > 0.05$). Likewise, no significant correlation was observed between i.v. saline self-administration and subsequent ethanol consumption (g/kg) for animals in saline group 2 ($r = -0.02$, $p > 0.05$). Nor was the correlation significant for saline group 1 animals ($r = 0.540$, $p > 0.05$), although the value of r is reasonably large. The significant positive relationship shown between acetaldehyde self-administration and subsequent amount of ethanol consumed by animals in acetaldehyde group 1 but not in acetaldehyde group 2 is consistent with the suggestion that the effect of acetaldehyde is dependent on body weight reduction. A similar trend observed in rats in saline group 1 would suggest that food deprivation alone may induce alcohol consumption. Caution concerning the use of the correlation coefficient to infer causal relationships between variables has been noted by other investigators[13].

Results of this investigation indicate that rats with a history of acetaldehyde self-administration show greater consumption of and preference for alcohol than animals allowed to self-administer saline; the effect being much greater when coupled with conditions of food deprivation than under free-feeding conditions. The evidence for acetaldehyde playing a role in an increased ethanol preference thus agrees with suggestions made by other authors on the basis of analogous experiments[10]. It is possible that the method of schedule-induced self-injection of acetaldehyde used in the self-administration phase of this experiment could have contributed to the maintenance of sufficiently high circulating acetaldehyde levels to induce other acetaldehyde-mediated mechanisms to become active. Circulating acetaldehyde is known to have a half-life of only a few minutes because it is rapidly destroyed by acetaldehyde dehydrogenase (ALDH)[14], the enzyme responsible for the oxidation of acetaldehyde to acetate[14]. Since the animal could voluntarily

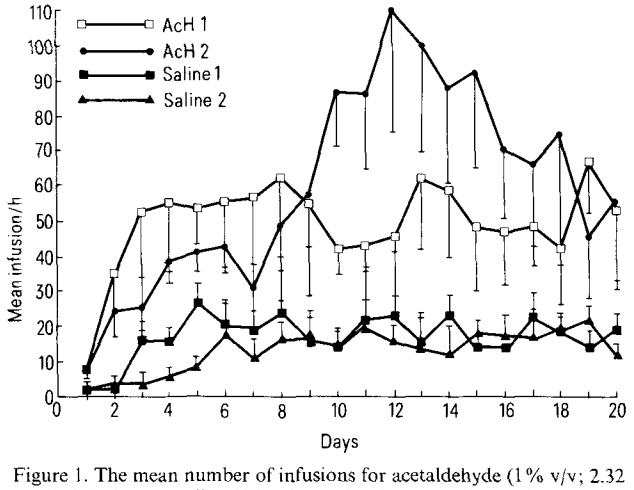

Figure 1. The mean number of infusions for acetaldehyde (1% v/v; 2.32 mg/kg infusion) or saline SEM, for each 1-h test session of the 4 groups of animals.

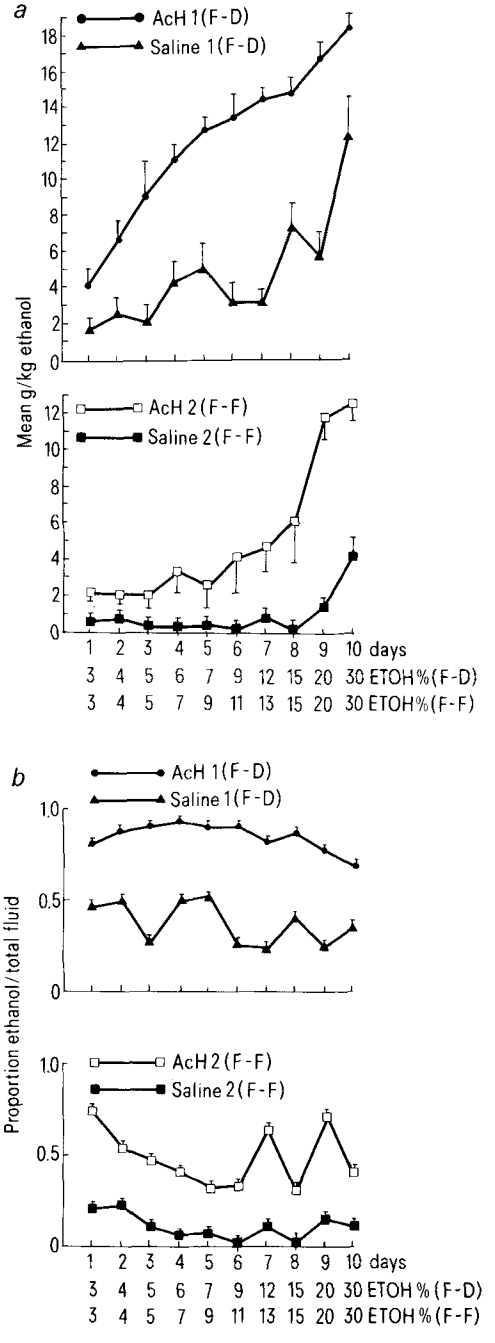

Figure 2. *a* Mean g of ethanol per kg b.wt consumed by each group of rats for each concentration of ethanol offered during food-deprivation (F-D) and free feeding (F-F) conditions. *b* Mean proportion of ethanol to total fluid intake consumed by each group of rats for each concentration of ethanol offered during the 10-day preference sequence.

self-administer multiple doses of acetaldehyde and was limited only by the 5-sec delay incorporated into the drug delivery system, this suggestion seems plausible. The report that food deprivation in combination with exposure to ethanol reduced blood-brain barrier functions[15] is interesting in this context. These suggestions do not eliminate the possibiliy that withdrawal of a reinforcing substance, acetaldehyde[1,9,16] for 2 days prior to the start of the ethanol preference sequence, may have led to increased intake of another drug, ethanol.

We believe these results to be important for several reasons. First, they show that the voluntary intake of a metabolite can shift the preference function for its precursor ethanol, one of the most widely used drugs. Secondly, since acetaldehyde changes ethanol preference, the results suggest a strong prima facie case for a) the possible in vivo synthesis of an alkaloid which perpetuates alcohol intake, and/or b) acetaldehyde-induced stimulation of catecholamine release that mediate consummatory functions and reward; the mechanism controlling the release may be linked to the function of aldehyde metabolizing enzymes, particularly ALDH[14]. Thirdly, the results suggest that ethanol preference in the rat may be affected by an association between (a) and/or (b) and possible nutritional variables[10,17-19]. Fourthly, the results suggest the need for an awareness of the possibility that acetaldehyde (which is found to be more abundant in wines than beers and distilled spirits[20]) induced effects may have to be considered separately from that of ethanol when assessing health problems related to high intake of alcohol beverages.

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Antifeedant nature of the quinone primin and its quinol miconidin from *Miconia* spp.

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Summary. The quinone primin (**1**) and its quinol miconidin (**2**) which occur naturally in Miconia spp. (Melastomataceae), were synthesized and then tested as potential antifeedants against 6 insect species. Antifeedant activity was found in all cases, ranging from primin (**1**) being most active against *Pieris brassicae*, to miconidin (**2**) being only slightly effective against *Heliothis armigera*.
Key words. Miconia spp.; quinones; primin; miconidin; anti-feedant activity, insect.

The presence in higher plants of quinones and of their reduced forms, quinols, is generally associated either with the process of cellular respiration and photosynthesis[2] or with their defence against insects[3] or with allelopathy[4].

Primin (2-methoxy-6-pentyl-1,4-benzoquinone, **1**), found in the leaves and in the glandular hairs of *Primula obconica*[5], causes severe dermatitis and allergy in some individuals[6]. Primin (**1**) was also isolated, together with its quinol miconidin (**2**), from *Miconia* species[7].

We therefore considered it to be of interest to establish whether compounds **1** and **2**, which are highly active biological substances[6-8], could also be involved in a mechanism for the protection of plants against some insect species.

Methods. Owing to the difficulty of obtaining the compounds investigated from natural sources in amounts sufficient for biological tests, large amounts were prepared by simplification of a known route[9].

Thus o-vanillin (**3**) in Et_2O was treated at 0 °C with n-BuLi (2 eq.) to give after a standard work-up procedure the alcohol **4**[10] in 95% yield (b.p. 112 °C/8 mm Hg; ^{1}H-NMR ($CDCl_3$+TMS): δ 3.85 (s, 3H), δ 6.70 (s, 3H); IR (CCl_4): 3540–3400 cm^{-1}.

The latter compound (5.85 g, 24.8 mmoles) was hydrogenated in EtOH (50 ml) for 2 h at room temperature and atmospheric pressure in the presence of 10% Pd(C) (500 mg) and 2N H_2SO_4 (0.2 ml), affording in 65% yield compound **5**, which had previously been prepared from **3** in 5 steps[9].

Compound **5** was then converted into primin (**1**)[11] by Fremy's