After we have determined the chemical structure of this female specific aphrodisiac, we will be able to investigate its relation with the young male sex appeal, the site and mechanism of its emission, and look for mutations that may affect either its emission or detection.

1 Acknowledgments. Thanks are due to Drs Y. Hotta, S. Benzer and C. Descoins for stimulating discussions at the beginning of this work. Most of it was performed in the Centre de Génétique Moléculaire du C.N.R.S. We want to acknowledge their financial support.

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Determination of odour affinities based on the dose-response relationships of the frog's electro-olfactogram[1]

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Summary. Electro-olfactograms (EOG's) recorded from the frog's olfactory epithelium for 11 substances were used to calculate dissociation constants which in turn serve as an index for the affinity between odorant and receptor site. These constants were calculated with and without a correction for the odour partition between water and air. For a homologous series of 7 n-alcohols these values decrease up to 1-heptanol. The dose-response relationships were based on the peaks of the EOG's since the peak/plateau-ratio was concentration-dependent for some of the substances.

Ottoson[3] compared the odorous strengths of a series of n-alcohols using the amplitudes of the peaks of the electro-olfactograms (EOG's) evoked by equimolar concentrations and using vapour concentrations of an equal stimulative effectiveness. In the present experiment dissociation constants for 11 odorous compounds, 7 of which are aliphatic n-alcohols, have been calculated using Beidler's taste equation[4]. The interaction between the assumed receptor R and the stimulus S can be written as follows:

$$S + R \leftrightharpoons SR. \quad (1)$$

The amplitude of the response r depends on the odour concentration c as follows:

$$c/r = 1/r_m (c + K_D) \quad (2)$$

in which r_m is the maximum response for the interaction between the receptor involved and a given stimulus; K_D is the dissociation constant. The latter value is given by the intercept of the concentration axis in Beidler's plot[4,5].

Tucker[6] and Poynder[7,8] found that such a representation can describe the concentration dependence of the odour-receptor interaction as reflected in the amplitude of the EOG. In this paper this concept is further developed.

In order to do so, the following implicit assumption has been made: the interaction between stimulant and receptor is at equilibrium when the peak of the EOG has been reached. The characteristic shape of the EOG consists of a peak and a plateau level. Since the latter level does not always have the same characteristics with respect to odour concentration, as will be demonstrated in this paper, this plateau level has not been used to describe the odour-receptor interaction.

Materials and methods. All frogs (n = 18) used for the present experiments were pithed. They belonged to the Dutch varieties of *Rana esculenta*[9]. Odorous chemicals of the purest grades commercially available were used as stimuli (BDH, Poole, Great Britain; Fluka, Basel, Switzerland). The odour concentrations have been calculated from the saturated vapour pressures[10].

Further preparation of the frogs and recording methods were standard. For the odour application a specially designed 'air-dilution' olfactometer was used[12]. To a relatively vast continuous air flow, serving as a carrier, controlled quantities of odorous stimuli can be added. In this way the occurrence of pressure pulses on the epithelium is prevented, since addition of odorant does not change the velocity of the flow reaching the epithelium surface. The olfactometer delivers stimuli in dilutions from 2 up to 100 times with respect to the saturated vapour. Stimuli lasted 5 sec, with interstimulus intervals of 3 min. The experiments were carried out at 22 ± 1 °C.

Results and discussion. Ratios of the amplitude of the peaks and the plateaus of the evoked EOG's were constant for acetone (figure), thus agreeing with previous observations on butyl acetate, amyl acetate, 1,8-cineole and linalool[7,8]. However, for 1-hexanol this ratio increased with the odour concentration (figure). For this substance peak and plateau overlap at low concentrations (the peak-plateau ratio approaches one). In contrast, for cumene the peak-plateau ratio decreases slightly with increasing odour concentration (figure). Other experiments in our laboratory confirmed these observations for acetone and indicated a slight increase for m-xylene (Sluyter, unpublished observations). These results show that peak and plateau levels of the EOG have not necessarily a similar dose-response relationship for the various concentrations. This suggests that at least 2 processes are involved in the generation of the EOG. The nature of the proces(ses) other

than the odour-receptor interaction as well as the location of the generation of these other processes is unknown. It might be that non-specific adsorption to other cells e.g. supporting cells is reflected in a repolarization of the initial electrical signal coming from the bipolar receptor cells as reflected in the peak.

The amplitudes of the peaks of the evoked EOG's were measured as a function of the odour concentration and plotted in Beidler[14] plots. The plots gave a linear relationship between the odour concentration and the EOG amplitude ($r > 0.90$) for all tested substances. The dissociation constants for these substances extrapolated from Beidler plots are presented in the table. They range from 10^{-8} M to 10^{-3} M and show a minimum at 1-heptanol for the n-alcohols as a function of chain length. This indicates that 1-heptanol is the most effective stimulus in this series. For the n-alcohols the dissociation constants are approximately proportional to the vapour concentrations which evoke EOG's of the same amplitude[3]. However, these results differ slightly from the findings of Higashino et al.[13], who found 1-hexanol to be the most effective stimulus. These workers used water as an odour solvent, whereas Ottoson[3] used oil. Psychophysical threshold determinations on the n-alcohols using an 'air-dilution' olfactometer also showed 1-heptanol to be the most effective stimulus[14], which agrees with the present results. Besides the dissociation constants based on the odour concentrations in air, the table presents the dissociation constants based on the odour concentrations in water. For calculation of these values the following formula has been used:

$$\text{Log}\,K_{DW} = \text{Log}\,K_{D_A} + \text{Log}\,K_{W/A} \qquad (3)$$

in which $\text{Log}\,K_{DW}$ is the dissociation constant in water, $\text{Log}\,K_{D_A}$ the dissociation constant in air, and $\text{Log}\,K_{W/A}$ the partition coefficient water/air. The latter values are either from published data[15] or calculated[10,16]. After this correction the logarithms of the dissociation constants show a nearly linear relationship with the number of C-atoms of the alkyl chain for the n-alcohols. This relation is similar to the ones found for the chemotactic response thresholds in various eukaryotic organisms[17] and to human olfactory thresholds[18]. The corrected K_D-values for the other compounds of the table also show some similarity to the corrected human olfactory thresholds for these compounds. The logarithms of these thresholds are respectively (also in moles/l): cyclohexanol: −5.4, acetone: −3.9, cumene: −9.0, m-xylene: −7.7. The difference between the olfactory thresholds and the K_D-values is approximately 10^4–10^5. Moreover, the K_D-values found for the n-alcohols in this study are rather similar to the ones for n-alcohol anesthesia as a function of the uptake in erythrocyte ghost membranes[19].

The range of the K_D-values in the table suggests that binding of the stimulus to the olfactory receptor is less tight than for example hormone-receptor interaction[20]. The present values are rather similar to the ones determined from electrophysiological responses to a number of carbohydrates in taste reception[5,21]. However, a biochemical study using radio-active amino acids as ligands in a rainbow trout taste receptor preparation showed that for these amino acids binding sites exist which have higher affinities than the ones found here[22]. Furthermore, the present K_D-values alter gradually rather than abruptly with chain length (table). This gradual change with chain length and the range of the K_D-values suggest that the odour reception of n-alcohols involves rather nonspecific, probably hydrophobic, interaction as has also been suggested by various other authors[17,18,23].

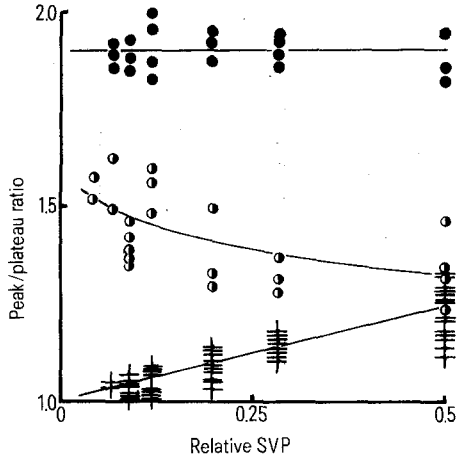
Values for the maximum response (r_m in formula (2)) show a large standard deviation for the odorous substances used. This is due to the large difference in the absolute amplitudes of the EOG's recorded in the various experimental sessions. For this reason these values are not presented here. At this stage these r_m-values do not make it possible to discriminate between a receptor system having only 1 receptor species or more than 1 receptor species for the various odorous compounds used.

From the present work it may be concluded that affinities of the olfactory receptors are adequately described by the dissociation constants as determined from the dose-response behaviour of the peak of the electro-olfactogram. These dissociation constants allow proper comparisons with physiological data obtained via other ways and/or in other systems[17–23].

Dissociation constants for the interactions between odorant and receptor without and with a correction for the partition of the odours between water (mucus) and air

Stimulus	Log K_D*	Log K_D corrected**	Number of Beidler plots used for the means	Number of frogs
1-Propanol	−4.0±0.3	−0.2	6	4
1-Butanol	−4.8±0.2	−1.4	14	7
1-Pentanol	−6.0±0.4	−2.6	10	6
1-Hexanol	−6.5±0.3	−3.3	18	11
1-Heptanol	−7.2±0.2	−4.2	6	5
1-Octanol	−7.0±0.3	−4.0	11	6
1-Decanol	−6.8±0.2	−4.8	4	2
Cyclohexanol	−5.2±0.3	−1.1	3	2
Acetone	−3.2±0.4	0.5	14	7
Cumene	−5.0±0.2	−3.8	12	7
m-Xylene	−4.4±0.4	−4.5	3	2

* K_D = dissociation constant (moles/l); these are presented with the standard deviations of the Log-values. ** K_D corrected = the dissociation constant after correction with the partition coefficient $K_{W/A}$ (formula 3).



Peak-plateau ratios of the electro-olfactogram for various odorants as a function of the odour concentrations. The maximum concentration on the abscissa is half the saturated vapour pressure (0.5×SVP) of the odorant in question. The plots are not drawn until they reach the ordinate since the value 0 indicates that no odorant is added. Every dot or dash indicates an EOG measurement. The following odorous substances were used: acetone (●——●) cumene (◑——◑), and 1-hexanol (+ — +).

1 This work was supported by Z.W.O. grant number 12-24-12 of the Dutch Government.
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The effect of endotoxin on plasma α-aminoisobutyric acid[1]

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Summary. The i.v. injection of bacterial endotoxin into dogs was found to cause a rapid increase in plasma levels of infused α-aminoisobutyric acid. The findings suggest that nonmetabolic factors (tissue uptake, fluid shifts) influence amino acid distribution during endotoxemia.

Altered nitrogen utilization and distribution are among the most critical consequences of infection with plasma amino acids generally declining in the afebrile state even before the onset of anorexia[2,3]. The metabolic response to early sepsis may differ from prolonged infection since i.v. injection of live *Escherichia coli* bacteria in dogs produces a rapid and pronounced elevation of alanine and other amino acids[4]. This increase has been attributed to a reduced hepatic conversion of alanine to glucose. The injection of *E. coli* endotoxin into dogs also increases plasma alanine levels but gluconeogenesis from U-^{14}C-alanine was not diminished after 4 h of endotoxemic shock[5]. To determine if nonmetabolic factors are involved in redistributing amino acids in early endotoxemia, 1-^{14}C-α-aminoisobutyric acid (AIB) was infused into endotoxin-treated dogs. This inert amino acid analogue is not incorporated into protein or otherwise metabolized but enters into cells by the same carrier system that transports alanine[6,7]. No physiological action or transmitter function has ever been reported for tracer amounts of AIB; its metabolic inertness and very slow penetrance into brain has been confirmed many times[8].

Materials and methods. Overnight fasted dogs of either sex, 15-20 kg, were anesthetized with Nembutal (30 mg/kg) and infused with 1-^{14}C-α-aminoisobutyric acid (AIB) (Amersham-Searle, Arlington Heights, IL.) in sterile saline at the rate of 0.58 ml/min. Each ml contained 0.15-0.20 mCi of AIB plus 0.66 mg added carrier AIB. An LD_{70} dose of *E. coli* endotoxin (Difco, Detroit, MI) was given i.v.

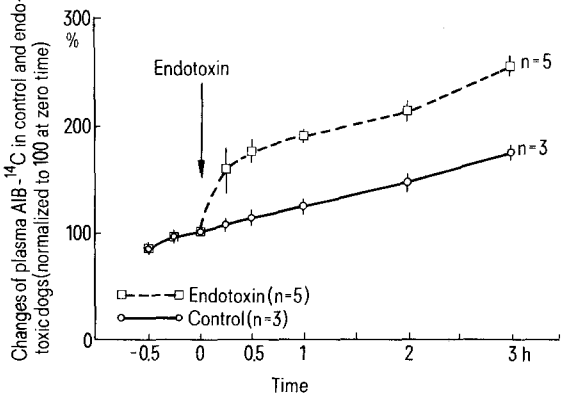

Fig. 1. The increase of plasma levels of infused α-aminoisobutyric acid after endotoxin injection. Vertical lines, mean ± SEM.

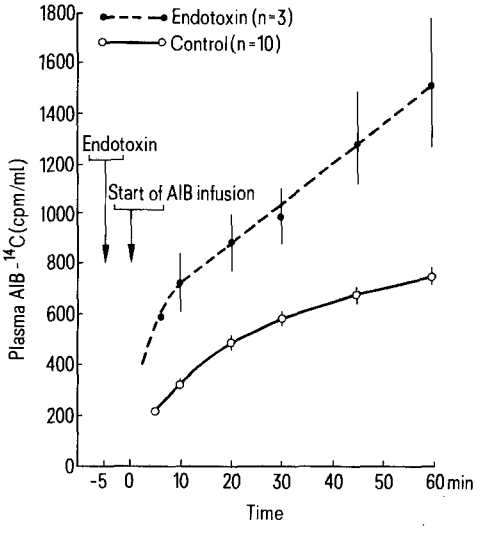

Fig. 2. The increase in plasma counts of α-aminoisobutyric acid when dogs are pretreated with endotoxin before AIB infusion. Vertical lines, mean ± SEM.