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The Response of the Honeybee Antennal CO_2-Receptors to N_2O and Xe*

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Summary. The response properties of the antennal CO_2-receptors in workers of *Apis mellifera* L. were studied electrophysiologically by extracellular recordings from single cells.

1. For a proportion ($>50\%$) of the receptors, the absolute sensitivities to CO_2 are higher than previously observed.

2. The spontaneous spike discharge and the response to CO_2 are reversibly inhibited by the inert gases N_2O and Xe; N_2O has a marginally stronger effect than Xe.

3. For a gas mixture containing a given CO_2-concentration above threshold the inhibition consists primarily of an increase in the response latency which is found to be proportional to the inhibitor concentration.

4. For a given concentration of N_2O or Xe, the magnitude of this effect decreases proportionally with an increase in CO_2-concentration.

5. A simple empirical equation for the latency as a function of the concentrations of excitatory stimulus and inhibitor is derived from the data.

6. It is investigated to what extent the experimental findings are consistent with kinetic models based on the law of mass-action.

7. It is suggested that the inhibition occurs via molecular ordering effects in the aqueous or lipid phases of either the cell membrane or the surrounding medium.

A. Introduction

The aim of the present work is to investigate the electrophysiological responses of the honeybee antennal CO_2-receptors to excitatory and

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inhibitory stimuli and to derive quantitative information on the molecular mechanism of CO_2-reception from the presented data and from related studies in the fields of general anaesthesia, enzyme mechanisms and chemical kinetics.

Some of the excitation properties of the honeybee CO_2-receptor have been previously investigated electrophysiologically (Lacher, 1964) and in behavioural experiments (Lacher, 1967). In a theoretical paper (Diesendorf *et al.*, 1973) we chose this receptor as a test case for the hypothesis that modal radiation mechanisms are involved in primary processes of chemoreception, and showed experimentally (unpublished data), that neither this receptor nor the honeybee H_2O vapour receptor could operate by detecting infrared emission or absorption lines from stimulus molecules.

Any theory attempting to clarify the mechanism of operation of a chemoreceptor must account for the observed sensitivity, the specificity of excitation and inhibition, and for the delay time of the responses to onset and cease of stimulation. To obtain information on the specificity, one can modify certain properties of the stimulus molecules, such as the length of a hydrocarbon chain and the number of side-chains (Mullins, 1955; Dumpert, 1972; Kafka, 1970, 1972), the configuration of double bonds (Schneider *et al.*, 1967), the position of heteroatoms and hydrogen isotopes (Doolittle *et al.*, 1968; Hummel *et al.*, 1972), the molecular shape (Amoore *et al.*, 1964; Amoore, 1971) or the infrared spectrum (Dyson, 1937; Wright, 1954; Wright and Brand, 1972; Wright, 1972), and determine correlations between such properties and the response of receptors.

The carbon dioxide molecule is one of the simplest possible chemical stimuli with well known properties, and one of the most common metabolites. There are, however, only limited possibilities for the outlined approaches: one possibility is to test the response of a CO_2-receptor to N_2O, which is similar to CO_2 in its stereochemical and physical properties. Xe was tested because any observed alteration of the CO_2-response eliminates any mechanism not involving the simple physical interactions which are possible for a monoatomic gas.

B. Materials and Methods

Workers of *Apis mellifera* L. were anaesthetized with CO_2 and attached to a holder covered with a beeswax-resin mixture; to exclude any after-effects of narcosis (cf. Lacher, 1964), the animals were permitted to recover for at least 30 min before recordings were started. The antenna was kept in an appropriate position by a piece of thin plastic tubing which was slipped across the pedicel-flagellum-joint and attached to the holder. After the indifferent electrode was

inserted into the last segment, the antenna was sufficiently immobilized to permit single cell recordings. The electrodes were electrolytically sharpened tungsten needles. By inserting the different electrode into the cuticle close to the position of the pit organs (cf. Lacher, 1964) spike potentials from CO_2-sensitive cells could be obtained. The signals were *AC* coupled into a Grass P 16 preamplifier and processed by standard methods.

Disposable polyethylene syringes (cf. Kafka, 1970; Vareschi, 1971) were found to provide the best method for stimulation. The syringes were filled with the gas mixtures immediately before use to minimize diffusion effects in polyethylene. They were connected via a valve to a steel tube of 0.9 mm internal diameter whose tip was brought as close as possible (1–1.5 mm) to the recording site. The valve consisted of a piece of thin silicone rubber tubing, which could be blocked by an electromechanically operated clamp. Tension from a spring was applied to the syringe piston to compress the contents to 1.5 atmospheres.

By operation of the valve 5–8 stimuli of 500 msec duration and intervals of 5 sec were obtained per syringe filling of 10 ml. The first two stimuli were used to permit the system to reach equilibrium and were not recorded.

A comparison of the responses to subsequent stimulations permits one to estimate the accuracy of the method: often the spike trains within a series were almost identical; for high CO_2-concentrations, the time between activation of the valve and occurrence of the first spike varied less than ± 2 msec. As the receptors themselves were the fastest CO_2-sensing devices available for the present study, the latency measurements below contain an unknown but constant contribution resulting e.g. from relay closing times and the time needed for the gas current to reach the receptor; from the fastest observed responses this contribution can be estimated to be < 40 msec.

Under the given conditions the gas current is ejected from the tube tip with a velocity of approximately 3 m/sec. In addition a second current of CO_2-free air at 30 cm/sec was permanently passed across the preparation via a tube of 6 mm diameter in a coaxial position to the stimulating tube.

The stimulus gases were prepared by mixing known volumes of high purity N_2O or Xe with various CO_2-air-mixtures of known CO_2-concentrations. An infrared gas analyser URAS 2 (Hartmann und Braun) with a sensitivity better than 10 ppm CO_2 in combination with a cascade of gas mixing pumps (Wösthoff) was used for calibration and for purity tests.

C. Results

1. The Response to CO_2 Stimuli

The CO_2 response characteristics observed in this study do not confirm some of the findings by Lacher (1964). He reports that the resting activity in room air was not or only negligibly influenced by stimulation with CO_2-free air, pure N_2, pure O_2 or mixtures of these gases. Kellog (1970) found in electrophysiological experiments that CO_2-receptors on the antenna of *Aedes aegypti* show different responses to 0.04 and 0.05%, and according to Lacher (1967) bees can distinguish e.g. between 1 and 3% CO_2 and between 35 and 50% CO_2 in behavioural experiments.

In contrast to this, we find that a proportion of the receptors responds with a noticeable increase in spike frequency to stimulation with a gas

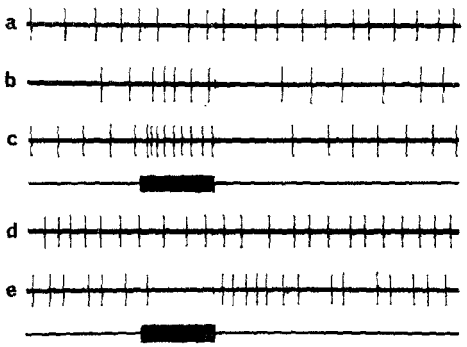

Fig. 1a—e. Responses of a CO_2-receptor, which was exposed between stimulations to CO_2-free air (a—c) or to air containing 10^{16} molecules CO_2/ml (d—e). Stimulation with (a): CO_2-free air (control); (b): 10^{15} molecules CO_2/ml; (c): 10^{16} molecules CO_2/ml; (d) 10^{16} molecules CO_2/ml (control); (e): mixture of gas from same sample as (d) with 10% CO_2-free air. Stimulus markings: 400 msec

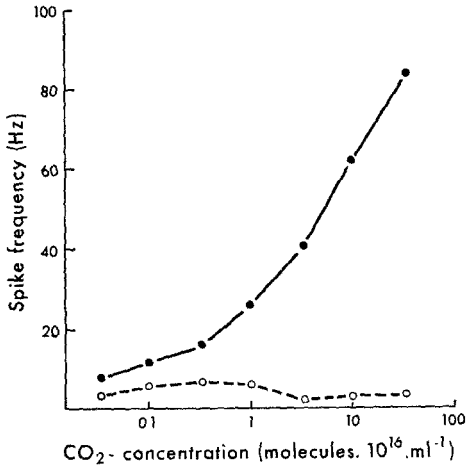

Fig. 2. Response characteristics of a CO_2-receptor to CO_2-stimuli in the range about the normal CO_2-concentration in air of 10^{16} molecules/ml. ●——● spike frequency during first 300 msec of stimulation; ○----○ spike frequency during last second before onset of corresponding stimuli. Arithmetic means from 3–6 measurements for each point

mixture containing the normal CO_2-concentration in air (10^{16} molecules/ml; Fig. 1c) and to even lower CO_2-concentrations (Fig. 1b, 2). The method used here becomes uncertain at 10^{14}–10^{15} molecules/ml; from

the observations it can be estimated, however, that the lowest occurring thresholds are below 10^{14} molecules CO_2/ml.

As already reported by Lacher (1964), the absolute sensitivity of individual cells varies greatly; within a sample of 20 cells, we found that 12 responded to stimulation with 10^{16} molecules/ml with an increase in spike frequency at least as noticeable as for the example in Figs. 1 and 2. The highest observed threshold was above $3 \cdot 10^{17}$ molecules/ml.

The CO_2-receptors also have a remarkable difference sensitivity: when we replace the CO_2-free air used for the conditioning current by air containing 10^{16} molecules/ml, we find that the steady state discharge is not influenced by stimuli with air from the same sample (Fig. 1 d), but that a reduction in the CO_2-content by 10% is sufficient to stop the activity for the time of stimulation (Fig. 1 e). Under the same conditions, air samples from different parts of the laboratory and from outside influenced the discharge in different ways dependent on their sites of origin, presumably due to slight variations in CO_2-content.

In most of the cells, a resting discharge of a few Hz (Figs. 1 a, 2) was observed under CO_2-free conditions; however, in a fraction of about 20% of the cells including highly sensitive ones such a discharge did not occur.

In the experiments the animals were breathing normal room air, and as in addition to this some metabolic CO_2 is produced inside the animal, the CO_2-concentrations inside the receptor cells may be considerably higher than the observed threshold concentrations. Therefore it is possible that the resting activity, where observed, is influenced by leakage of this metabolic CO_2 towards a hypothetical acceptor site within the receptor cell membrane.

2. The Inhibition by N_2O and Xe

General. If a CO_2-receptor is stimulated with N_2O, the resting discharge is inhibited during stimulation and a phasic rebound occurs after termination of the stimulus. The magnitude of this rebound increases with the inhibitor concentrations (Fig. 3, top row). The response follows a time course which is similar to the response to CO_2, except for the sign (Fig. 4 a, b), but much higher N_2O-concentrations are needed to get an obvious effect. For stimulation with Xe the same kind of response is observed, but it is marginally weaker (see Figs. 5 and 6).

In this context the terms "inhibition" and "inhibitor" refer to the suppression of spike activity on application of a stimulus, which is presumably caused by hyperpolarization of the receptor cell membrane; it has to be distinguished from an inhibition on the molecular level, e.g. of enzymes, although it may be related to it (see below). Until a more detailed knowledge on the mechanism of inhibition is

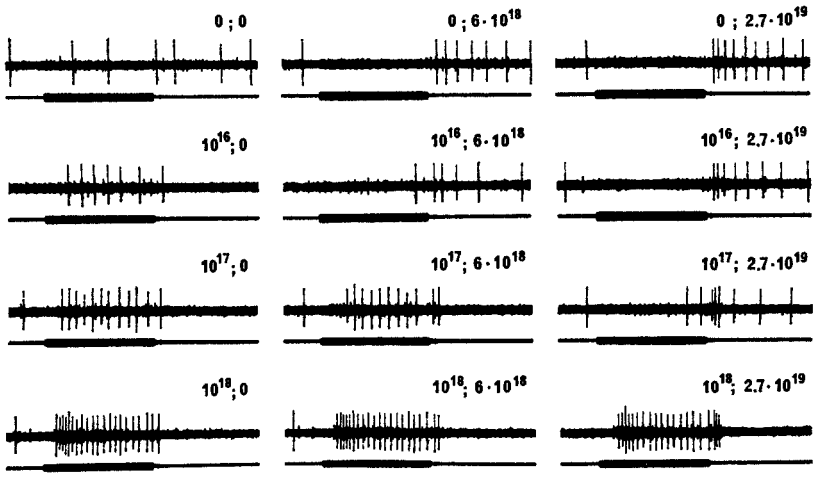

Fig. 3. Response of a CO_2-receptor to increasing CO_2-concentrations (columns) in combination with increasing N_2O-concentrations (rows). First figures: CO_2-concentrations; second figures: N_2O-concentrations in molecules/ml. Stimulus markings: 500 msec

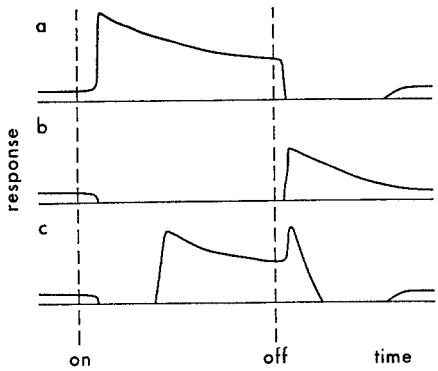

Fig. 4a—c. Schematic presentation of observed characteristics. Response magnitude versus time. (a) CO_2 stimulus. (b) N_2O or Xe stimulus. (c) Combination of (a) and (b)

available, we maintain the general term which is commonly used for similar effects in related work (e.g. Boeckh, 1967; Kafka, 1971; Vareschi, 1972).

When searching for a CO_2-receptor, spontaneous spike activity was frequently recorded from cells which were not sensitive to CO_2; no N_2O-effect has so far been observed in any of these cells. On the other

hand, an inhibitory effect of N_2O was observed in all cells which also responded to CO_2, whereas Argon, N_2 and O_2 were not effective.

Not only the CO_2 response properties—threshold (see above), latency, slope of the response curve and phasic component—vary considerably between individual cells, but also the magnitude of the inhibitory effect varies.

When a N_2O stimulus is applied together with a CO_2 stimulus (Figs. 3 and 4c), we find that the spontaneous discharge is inhibited after stimulus onset, but after a delay dependent on both the CO_2- and the N_2O-concentration the cell begins to fire in response to the CO_2 stimulus. As no spontaneous spikes occur during the delay, this effect is qualitatively different from the increase in latency observed with decreasing CO_2-concentrations (Fig. 3, first column), i.e. there is a genuine inhibition, but for reasons of simplicity we refer to the time delay as a latency.

After the end of a combined stimulus, the effects are a combination of the transient poststimulus inhibition as observed for CO_2 alone, and the rebound effect of the inhibition (Figs. 3, 4); for high concentrations of both stimuli this after-effect typically consists of a group of three spikes with small invervals (Fig. 3, last recording).

Detailed Analysis. The quantitative relationship between excitation and inhibition was studied by stimulation with the possible combinations of eight CO_2-concentrations in 0.5 log unit steps with four N_2O-concentrations in 0.3 log unit steps and with one of Xe.

The Figs. 3 and 5–8 describe the response characteristics of an individual but representative cell: it had an intermediate sensitivity, and the effect of the inhibition was sufficiently pronounced to permit accurate measurements.

Fig. 5 shows the effect of various inhibitor concentrations on the latency as a function of CO_2-concentration. In the absence of inhibitor, the latency decreases with increasing CO_2-concentration; the slope in the logarithmic plot decreases slightly at high CO_2-concentrations.

For large inhibitor concentrations no spikes occur during the 500 msec of stimulation when the CO_2-concentration is low, and for intermediate CO_2-concentrations it becomes apparent that the latency is sharply increased. For high CO_2-concentrations the curves converge, indicating that the maximum response is independent on the inhibitor concentration. As in all other cells studied, the effect of Xe is slightly weaker than the effect of N_2O.

If the spike frequency after response onset is plotted in a corresponding way (Fig. 6), we find that the response in the absence of inhibitor depends logarithmically on the CO_2-concentration for more than two

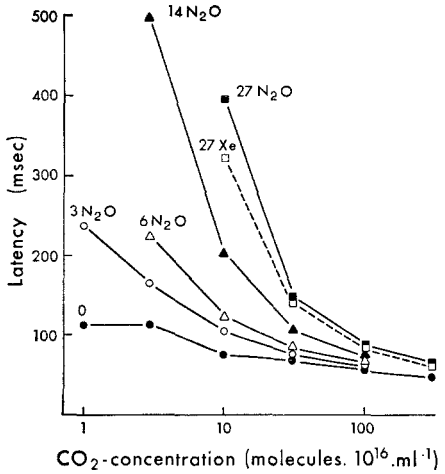

Fig. 5. Latency (time between stimulus onset and occurence of first spike) as a function of CO_2-concentration for various concentrations of N_2O or Xe. Data from same cell as in Fig. 3; medians from 3–6 measurements for each point. Figures in the plot give inhibitor concentrations in molecules · 10^{18} · ml^{-1}

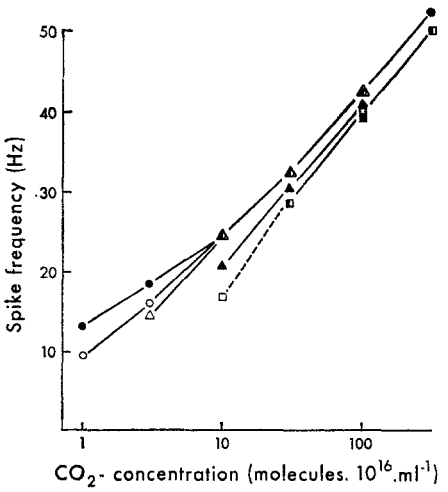

Fig. 6. Spike frequency (mean reciprocal spike intervals from 3–6 measurements for each point) after occurrence of first spike during stimulation. Same data and symbols as in Fig. 5; points are plotted only if a least three spikes occurred between response onset and stimulus end

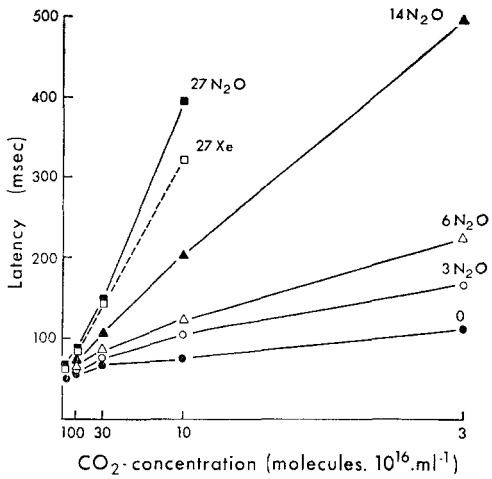

Fig. 7. Same data as in Fig. 5; latency as a function of CO_2-concentration, replotted on an inverse-concentration scale

log units. The influence of the inhibitor on the spike frequency is weak, and even this influence is uncertain because only a few spikes occurred at low concentrations and because the phasic-tonic character of the response (which was weak for this cell, see Fig. 3) was not considered, resulting in a systematic error. Nevertheless, the data are adequate to point out that the magnitude of the excitatory response is not influenced much once it has started, and that the essence of the inhibitory effect is a retardation in the response to the excitatory stimulus.

D. Quantitative Description

Latency. If we replot the data of Fig. 5 as a function of the reciprocal of the excitatory stimulus concentration S (Fig. 7) it becomes evident that for a given inhibitor concentration I the latency τ is linearly dependent on $1/S$:

$$\tau_I(S) = m(I) \cdot \frac{1}{S} + \tau_0. \tag{1}$$

In the same way, a plot of τ as a function of I for various values of S (Fig. 8) reveals that:

$$\tau_s(I) = n(S) \cdot I + d(S) \tag{2}$$

with the slope $n(S)$ being proportional to $1/S$; the intercept $d(S)$ is the sum of a constant and a term approximately proportional to $1/S$. Thus

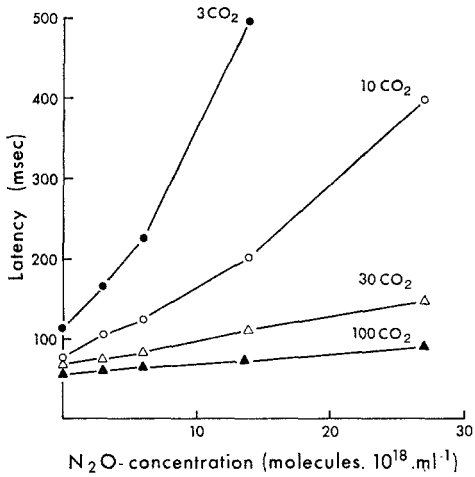

Fig. 8. Same data as in Fig. 5; replotted for latency as a function of N_2O-concentration. Figures in the graph give CO_2-concentrations in molecules $\cdot 10^{16}$ ml^{-1}

a complete empirical relation, expressing latency as a function of the two variables S and I, becomes

$$\tau(S, I) = a\frac{I}{S} + \frac{b}{S} + \tau_0 \tag{3}$$

where the positive constants a and b are characteristics of the particular receptors studied. The first term on the right-hand-side of Eq. (3) expresses the ratio of affinities of inhibitor and exciter to the receptor; the constant a has the dimension [time] and is obtained from the slopes of the curves in Fig. 8. The second term of (3) provides a rough fit to the behaviour of the latency in the absence of inhibitor; over the range of values of S studied, for several receptors, the behaviour is actually b/S^{α}, where α lies in the domain $\frac{1}{2} < \alpha \leqq 1$. The constant b has dimensions [concentration] $\times$ [time], and can be obtained directly from the slope of the $I = 0$ curve in Fig. 7.

The present set of experiments does not provide absolute measurements of latency, but rather measurements of changes in latency. The term τ_0 is the value of the latency in the limit $S \to \infty$, and includes such effects as time delays caused by the experimental method and the time between the generation of a receptor potential and spike generation.

For the responses of the receptor described by Figs. 3–8, the following values for the constants in Eq. (3) are obtained: $a = 1.0$ msec, $b \cong 1.5 \times 10^{18}$ msec $\cdot$ cm^{-3}, $\tau_0 \cong 50$ msec.

The equations exhibit algebraically the observation that in the absence of inhibitor, i.e. $I=0$, the latency varies approximately as the inverse of the exciter concentration S over two orders of magnitude. For large values of S the latency begins to show signs of tending to a limit. In the presence of both inhibitor and exciter, this behaviour remains unchanged, but there is an additional contribution to the latency which is directly proportional to the ratio of inhibitory and excitatory stimulus concentrations. The second term of Eq. (3) accounts for the different latencies observed for different values of S in the limit $I \to 0$ (see Fig. 8).

Spike Frequency. Fig. 6 shows that the spike frequency f is proportional to log S for all experimental values of I. The dependence of f on I is clearly very weak, except possibly at low values of S where the uncertainty in the data becomes large on account of the small number of spikes obtained.

E. Discussion of Mechanisms

The observed high differential sensitivity of the honeybee CO_2 receptors could be useful to the insect in controlling the ventilation of the hive (cf. Chauvin, 1968), for monitoring the physiological state of larvae, and for the recognition of photosynthetically active food plants via the detection of CO_2 gradients (cf. Lacher, 1964). The high absolute sensitivity to CO_2 and the observed effects of N_2O and Xe provide useful information on the receptor mechanism.

In an investigation of carrion beetle olfactory receptors, Boeckh (1967) applied an excitatory stimulus (carrion odor or aliphatic alcohols) simultaneously with an inhibitory one (propionic acid), and found that the responses follow different time courses and are additive. This suggests that both sets of stimuli operate on different molecular sites.

In the case of the honeybee CO_2-receptors, the difference in absolute sensitivity between excitation and inhibition, taken together with the apparent high specificity of excitation, leave open the possibility that specificity could be determined in different ways in excitation and inhibition.

The present experiments permit several mechanisms for the honeybee CO_2-receptors to be ruled out immediately:

1. Hydrogen Ion Concentration. Brown (1972) reports that the effects of CO_2 on neurons of *Aplysia californica* are due to pH changes. Under physiological conditions, only negligible pH changes would result from the CO_2-concentrations in the threshold range of the honeybee CO_2-receptor; therefore they cannot be of critical relevance here, unless an active transport mechanism is involved, which concentrates stimulus molecules at the site of action.

2. Infrared Radiation. According to detectability criteria developed in an earlier paper (Diesendorf *et al.*, 1973), the currently observed sensitivity to CO_2 is too high to be consistent with a mechanism involving the long range detection of radiant energy emitted or absorbed by the gas molecules. Furthermore, experiments performed with CO_2 in a sealed chamber with an IR-transparent window demonstrated that a radiation mechanism is ruled out independently of any choice of detectability criteria. Since N_2O and Xe have no common spectral lines or bands on the infrared, a radiation mechanism of inhibition is ruled out as well. In this context we mention that the honeybee antennal H_2O-receptor is also excited by the isotopic molecule D_2O, thus ruling out for this receptor any mechanism in which specificity is determined by a characteristic wavelength of molecular vibration or rotation; e.g. a resonant collision mechanism (Wright *et al.*, 1956, 1970) is also ruled out for the H_2O-receptor.

3. Molecular Shape. One initial reason for testing N_2O was its similarity to CO_2: the molecules are isosteric, have the same molecular weight, and similar electronic configurations, vapour pressure, solubility in water, etc.

The difference in the responses to CO_2 and N_2O, and particularly the fact that Xe as a monoatomic gas has the same effect as N_2O, show that shape alone is not important in determining specificity.

A common property of the three stimulus molecules is their small size; the lack of a response of the CO_2-receptors to 33 normal olfactory stimuli (Lacher, 1964), all larger molecules, supports the idea that the small size of the stimulus molecules is important. The stimuli also have in common zero or small (in the case of N_2O) dipole moments and, in comparison to ineffective stimuli such as N_2, O_2 and Ar, high polarizabilities. The above factors are of critical relevance for the specificity of adsorption processes and for the distribution e.g. in lipid-water interfaces such as cell membranes.

Xenon is known to behave as a general anaesthetic (Lawrence *et al.*, 1946), an effect it has in common with both CO_2, N_2O and other "inert" gases (Cullen *et al.*, 1951). Mullins (1955) studied the relations between molecular size, odour and anaesthetic activity; he proposed that Xe is odourless because there is a high probability that a Xe-atom attaches to a membrane site which is specific for larger molecules and narcotizes it, rather than finding a site of appropriate size (cf. also Beidler, 1961).

The site and mode of general anaesthetic action is not yet fully understood, but it is evident that molecular ordering effects acting on nerve cell membranes or the surrounding medium are involved. Pauling

(1961) and Miller (1961) proposed independently that the formation of solid clathrate hydrates of the gases in the aqueous phase of the nervous system are relevant. Stabilization difficulties make the existence of solid structures unlikely at room temperatures, but molecular ordering in the liquid state is possible: evidence for increased molecular ordering in aqueous non-electrolyte solutions is provided by the observed variation of molar volume, activity, sound velocity and free energy as a function of solute concentration (e.g. Glew *et al.*, 1968). Recent work (e.g. Lever *et al.*, 1971; Miller *et al.*, 1971) supports the theory that general anaesthetic effects occur in lipid regions of cell membranes. Clements and Wilson (1962) investigated the effects of anaesthetics on lipoprotein surface films covering water, and suggested that the essence of the reaction could be the formation of lipid-protein-water-agent "complexes" at interfaces within cellular membranes, and that therefore the difference between hydrate and lipid theories of inert gas narcosis becomes indistinct.

A comprehensive review on the membrane effects of anaesthetics was recently given by Seeman (1972).

Within this discussion the question is of particular relevance whether small inert molecules could possibly act on specific regions in protein molecules such as a hypothetical acceptor for CO_2. This possibility is suggested by known analogies:

In an investigation of the effects of anaesthetics on luminous bacteria, Halsey and Smith (1970) conclude that the most likely mode of action is a specific and reversible inhibition of the luminescent system, possibly through interactions with reduced luciferase. Hegemann and Featherstone (1969) report that xenon and cyclopropane reversibly inhibit the growth of *Escherichia coli* in a mineral medium and they find that the anaesthetics act on certain dehydrogenases.

According to Riepe and Wang (1968) (but cf. Christiansen and Magid, 1970; Khalifah, 1971) N_2O and CO_2 have similar affinities to the CO_2-binding site of bovine carbonic anhydrase (carbonate hydrolyase, EC 4.2.1.1.). Riepe and Wang propose that this binding site is merely a hydrophobic surface or cavity which loosely binds non-polar molecules such as CO_2 and N_2O in preference to polar water molecules and ions.

There is some evidence that carbonic anhydrase might occur in cell membranes, and it is known to be involved, perhaps indirectly, in transport mechanisms for ions such as Na^+, K^+ and Cl^- (cf. Carter, 1972). These findings suggest that the molecular acceptor for CO_2 may have a binding site similar to that of carbonic anhydrase at which N_2O and Xe might bind competitively. If we regard latency as an inverse speed

parameter, the plot in Fig. 7 is formally comparable to a Lineweaver-Burk diagram, and indeed the data appear in a form which is consistent with a competitive mechanism. This poses the question whether simple kinetic models are adequate to describe the findings consistently.

F. Kinetic Models

The time courses of inhibition and excitation are similar (Figs. 3, 4); however the responses are not additive in the presence of both stimulus modalities (Fig. 4). Indeed, as illustrated by Eq. (3), a part of the observed latency depends on the concentrations of both the exciter and inhibitor, while another part depends on the exciter alone.

The results suggest:

a) that we are observing effects on at least two stages of the chain of processes which determine the response; this is supported by the finding that spontaneous spikes are missing during the period of latency for combined stimuli (Figs. 3, 4), and

b) that at one link in this chain the exciter and inhibitor act on the same site, or at least their effects combine before spikes, and presumably a membrane potential, are generated.

We are therefore led to consider, as a first step, a simple one-stage kinetic model of excitation and inhibition, in order to determine under what conditions, if any, it could provide a "fairly good" description of the observations, and where its limitations lie. We then investigate whether a simple two-stage kinetic model can remove these limitations.

Beidler (1954) first applied the law of mass action to the primary processes of chemoreception involving equilibrium reactions between stimulus and acceptor. Kaissling (1969) extended this work to the treatment of transients; e.g. to the calculation of formation and decay times for excitatory stimuli. We first compare our results with the simple one-stage reaction model discussed by Kaissling (1969).

Model 1. Stimulus molecules of concentration S interact reversibly with acceptors of concentration A to form a complex AS which in turn participates in a transduction process leading ultimately to the generation of spikes. The complex can also decay releasing a product P. The reaction is

$$A + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} AS \xrightarrow{k_3} A + P \qquad (4)$$

$$\Big\downarrow$$

spikes

leading, for the case of a step stimulus, to a linear first-order differential equation

$$\frac{dy}{dt} + \gamma y = k_1 S A_{\text{tot}} \tag{5}$$

with solution

$$y(t) = y_{\text{eq}}(1 - e^{-\gamma t}) \tag{6}$$

where $y(t)$ is the concentration of complex AS at the time t;

$$\gamma = k_1 S + k_2 + k_3 = k_1(K_{st} + S); \quad K_{st} = (k_2 + k_3)/k_1$$

is the steady state constant, and the plateau or equilibrium value of y is

$$y_{\text{eq}} = \frac{A_{\text{tot}} S}{K_{st} + S} = A_{\text{tot}} \cdot \frac{X}{1 + X} \tag{7}$$

and $A_{\text{tot}} = A + y$ is the total number of acceptors at the receptor cell, and is constant. The formation time τ_f, defined as the time for $y(t)$ to reach one-half the equilibrium value y_{eq} is given by

$$\tau_f = \frac{\ln 2}{k_1(K_{st} + S)} = \frac{\ln 2}{k_1 K_{st}} \cdot \frac{1}{1 + X}$$

where $X = S/K_{st}$.

Kaissling (1969) has compared y_{eq}, τ_f, and τ_d [cf. Eq. (9)] of the kinetic model with the amplitude, rise-time, and decline time respectively of the receptor potential observed in the queen substance receptor of the drone honeybee and in the EAG of the silkworm moth bombykol receptor. If the kinetic model is applicable to the production of spikes in the honeybee CO_2 receptor, then y_{eq} is a measure of spike frequency and τ_f is correlated with the latency (i.e. the time delay) for impulse response.

In the absence of inhibitor the observed latency, expressed as a function of S, follows the theoretical curve for the formation time [Eq. (8)] to a good approximation over two orders of magnitude of S. However the observed spike frequency varies as $\log S$ over 2 orders of magnitude of S, exhibiting no indication of saturation, while the theoretical curve for y_{eq} varies as $\log S$ over about one order of magnitude only, exhibiting both saturation at large S and linear behaviour at small S.

Thus, even for excitation alone, this kinetic model has shortcomings when applied to spike generation by the honeybee CO_2 receptor, and, in general, to any receptor exhibiting a log response over a range of several log units. However the model at this stage might still be considered to be "fairly good", because modifications could be made to extend the $y_{\text{eq}}(S)$ behaviour over a larger range of S.

The deviations from a Langmuir isotherm, as they are frequently observed in the responses of chemoreceptors, could be caused by electrical properties such as the relationship between stimulus-induced conductivity change and receptor potential (Morita, 1969; Kaissling, 1972) or the cable properties of a sensilla (Kaiss-

ling, 1971). However we also have to consider that molecular interactions or ordering at the membrane could be involved: Changeux *et al.* (1966) (cf. also Singer *et al.*, 1972) proposed that cooperative effects might occur between subunits of a cell membrane which lead to a change of substrate (stimulus) affinity as a function of the number of occupied sites.

In the presence of inhibitor, the model, if applicable, must exhibit no change, or a very slight change, in spike frequency, but a large change in latency which is proportional to I/S.

It follows from Eqs. (7) and (8) that this observation can be satisfied in two different ways:

(a) If the inhibitor operates by changing K_{st}, then we require $X \gg 1$, and so

$$Y \cong 1 - 1/X \tag{7a}$$

$$\tau \cong \frac{\ln 2}{k_1 K_{st}} \frac{1}{X} = \frac{\ln 2}{S} \cdot \frac{1}{k_1} \tag{8a}$$

where $Y = y_{eq}/A_{tot}$; i.e. in changing K_{st}, the inhibitor must primarily change k_1 and not $(k_2 + k_3)$.

(b) If the inhibitor operates by changing k_1 and $(k_2 + k_3)$ by the same factor, so that K_{st} and hence X is a constant under inhibition, then Y (i.e. the spike frequency) is a constant too, and τ_f varies as $1/k_1$. In this case there are no restrictions on the values of X.

The delay time τ_d at stimulus end is given by (Kaissling, 1969):

$$\tau_d = \frac{\ln 2}{k_2 + k_3} = \frac{\ln 2}{k_1 K_{st}}. \tag{9}$$

Hence if case (b) is operating, the formation time τ_f and decay time τ_d should both exhibit the same functional behaviour when I varies and S is kept constant. In case (a) we would expect τ_d to be independent of, or only very weakly dependent on, I. Sufficiently accurate data to distinguish cases (a) and (b) on the basis of poststimulus behaviour are not obtainable, but on a qualitative level it can be seen (Fig. 3) that the duration of the rebound after stimulus end increases with the inhibitor concentration and decreases with the exciter concentration.

There is a further observation which must be accounted for by any model; that in the limit $I \to 0$ there is a remaining contribution to the latency which is not a constant but a function of S; i.e. that in general there are two separate contributions to $\tau - \tau_0$ [Eq. (3)]. Here model 1 fails, but as we have seen, it does provide a fairly good description for the observation that inhibitor increases the latency but not the spike frequency.

We next investigate whether a better agreement between observations and kinetic formulation can be obtained from a two-stage kinetic model.

Model 2. A two-stage model is considered in which the complex AS, produced according to model 1, undergoes a conformational change to a state AS'. Spikes are now produced by a transduction process from AS'. Thus

$$\begin{array}{ccccc} A+S & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & AS & \xrightarrow{k_3} & A+P \\ & & \underset{k_5}{\overset{k_4}{\rightleftharpoons}} & & \\ & & AS' & & \\ & & \vdots & & \\ & & \text{spikes} & & \end{array} \tag{10}$$

A pair of simultaneous linear differential equations is obtained for the concentrations y and x of AS and AS' respectively:

$$\begin{aligned} &\frac{dy}{dt} + (\gamma + k_4)\, y - k_5\, x = k_1\, S A_{\text{tot}} \\ &\frac{dx}{dt} + k_5\, x - k_4\, y = 0. \end{aligned} \tag{11}$$

The solution is lengthy, so we simply state the principal result: that, although the inhibitor can now operate on the additional reaction $AS \rightleftharpoons AS'$, model 2 does not provide an improved description of the quantitative observations on excitation and inhibition.

G. Conclusion

The failure of simple one-stage and two-stage kinetic models to provide a complete description of both excitation and inhibition supports the hypothesis that we are not only observing two stages in the transduction process, but also that the interaction between those two stages cannot be described in terms of a straightforward competitive model.

Although it appears most likely that the inhibition involves molecular ordering resulting in a transient change in "fluidity" (e.g. a phase transition) of lipophilic regions in the cell membrane, which competes with the information flow from a specific excitatory input, we also have to consider some other possibilities.

For instance, the inhibition might occur more peripherally; the presence of high concentrations of inhibitor could cause a change in the diffusion rate of CO_2 from outside towards the site of events at the receptor cell membrane. Furthermore, we have to keep in mind that the high sensitivity to CO_2 and its excitatory effect in contrast to other stimuli may be due to its ability to react with water. Therefore, the actual

acceptor could be specific for hydrogen carbonate ions, and the inhibitors might act on a separate first step in the transduction chain involving a system with carbonic anhydrase activity.

It is a question of definition whether we regard the observed effects as olfactory ones or as somewhat specific anaesthetic ones (cf. the discussion of small molecule effects by Mullins, 1955): both approaches become indistinct if we deal with interactions on the molecular level. In any case, the next step must be the provision of an experimental basis for further conclusions; it can be expected that more useful information on sensory transduction mechanisms and on the mode of action of inert gas anaesthetics will be obtained from further studies on the CO_2-receptor.

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