combined filtrate and washing was concentrated and allowed to crystallize.

**p-[3,5-Dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonylthioureas (6 and 7).** A mixture of 1 or 2 (0.05 mol) and anhydrous potassium carbonate (0.1 mol) in dry acetone (100 mL) was stirred and treated with the appropriate isothiocyanate (0.06 mol). After stirring and refluxing the mixture for 10 h, acetone was removed under reduced pressure, and the solid mass thus obtained was dissolved in water and acidified with 2 N hydrochloric acid. The crude product isolated was purified by recrystallization from dilute ethanol.

**1-p-[3,5-Dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonyl-2-thiohydantions (8 and 9).** A mixture of 6 or 7 (0.01 mol) and ethyl bromoacetate (0.011 mol) in absolute ethanol (50 mL) was refluxed with stirring for 2 h, concentrated, and allowed to crystallize. The products obtained were recrystallized from ethanol.

**1-p-[3,5-Dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonyl-5,6-dihydro-4(3*H*)-oxo-2(1*H*)-pyrimidinethiones (10 and 11).** A mixture of 6 or 7 (0.01 mol) and ethyl 3-bromopropionate (0.011 mol) in absolute ethanol (60 mL) was refluxed with stirring for 2 h, concentrated, and allowed to crystallize. The products obtained were recrystallized from ethanol.

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# Differential Solubilities in Subregions of the Membrane: A Nonsteric Mechanism of Drug Specificity

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We studied the effect of four volatile anesthetics and convulsants on the GABA- and glutamate-induced conductance change in crab muscle. The intensity of drug action correlated with the *solubility parameter* ($\delta$) values of the four drugs. Thus, the higher that value was for a given drug, the stronger was its effect on the glutamate response but the weaker was it on the response to $\gamma$-aminobutyric acid (GABA). We suggest that different gating molecules are housed in specific subregions of the membrane, each characterized by a particular value of the solubility parameter. The differential distribution of drugs in these subregions may be a nonsteric mechanism for drug specificity.

It has been proposed that the volatile anesthetics and convulsants and perhaps other simple compounds act on the nervous system by dissolving in the hydrophobic core of the membrane, thus causing volume expansion and disordering of membrane lipids and possibly proteins.[1-5] This view dwells on the assumption that such drugs do not act on specific binding sites because they lack structural specificity and fail to show evidence for saturable binding to membranes.[6] Yet, the remarkable specificity of these agents in causing either anesthesia or convulsions has remained a challenge to the current theory. We have recently shown[7] that the potent convulsant fluothyl, $CF_3CH_2OCH_2CF_3$, blocks preferentially the response of the $\gamma$-aminobutyric acid (GABA) receptor in crab muscle fibers, whereas the anesthetic methoxyflurane, $CHCl_2CF_2OCH_3$, blocks preferentially that of the glutamate receptor in the same fiber. We now report on a correlation between the relative potency of four fluorinated ethers at each of these sites and their solubility parameters ($\delta$).[8,9] On the strength of this and earlier experimental[10,11] and theoretical[12] data, we propose a model for a nonsteric mechanism of drug specificity.

## Experimental Section

Experiments were performed on the adductor muscle fiber cells from the walking legs of the crab, *Ocypoda cursor*, in vitro. The procedure has been presented in greater detail in an earlier publication.[7] The preparation was continuously perfused with medium alone or medium containing the desired drug, at a temperature of 22 ± 2 °C. Muscle cells were impaled with two microelectrodes, one for voltage recording and the other for current injection. Hyperpolarizing current pulses of 100–500 nA for 100–200 ms were used to measure the effective input impedance of the cells. The voltage, $V$, and current, $I$, traces were suitably amplified and recorded. Membrane conductance, $G$, was computed as $G = I/V$ and was normally of the order of 100 kmho. The addition of glutamate ($5 \times 10^{-5}$ M) to the bath resulted in depolarization and a conductance increase 1.5 to 2.5 times that of the control. The addition of GABA ($5 \times 10^{-5}$ M) was not followed by a consistent change in membrane potential but induced a conductance change of the same order as that induced by glutamate. Typical intracellular recordings have been published earlier.[7] In addition to fluothyl and methoxyflurane, two more drugs of intermediate $\delta$ values have now been submitted to the same evaluation: fluroxene, $CF_3CH_2OCH{=}CH_2$ ($\delta = 7.77$), and enflurane, $CHFClCF_2OCHF_2$ ($\delta = 8.26$).

## Results and Discussion

There exists a correlation between the solubility parameter of a drug and its relative effect on the conductance change mediated by each of glutamate and GABA (Figure 1). The higher the value of $\delta$, the more powerful is the effect on the glutamate response but the weaker it is on the GABA response. The opposite is true for low $\delta$ values. This finding is consistent with earlier results implicating

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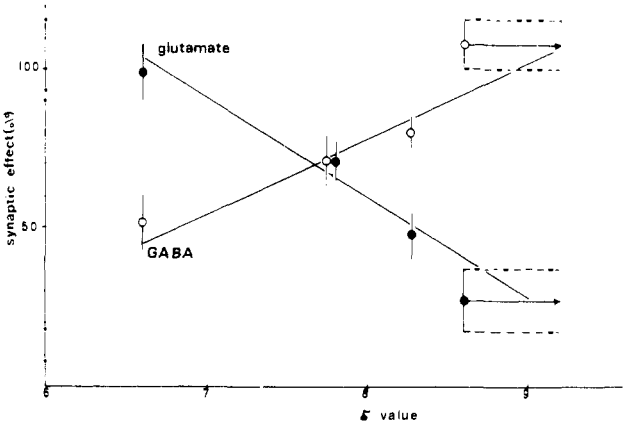


**Figure 1.** The correlation between the synaptic effect of four fluorinated ethers and their $\delta$ values. The ordinate shows the effect of either glutamate (closed circles) or GABA (open circles) on membrane conductance in the presence of $10^{-4}$ M of each of the four drugs. All conductance values are expressed as percentage of the control value without a drug. The abscissa shows the $\delta$ values $(cal/cm^3)^{1/2}$ of the four drugs:[10] fluothyl, 6.59; fluroxene, 7.77; enflurane, 8.26; methoxyflurane, 8.54–9.2. Bars indicate ±1 SD. The points for methoxyflurane represent eight experiments each and for the other two drugs three experiments each. The correlation coefficient for GABA was $r = \pm 0.94$ and for the effect on glutamate $r = -0.97$. Both correlations were significant at the 0.1% level.

$\delta$ in the modulation of the quality of drug action: Low $\delta$ fluorinated ethers are convulsants in animals while high $\delta$ ones are anesthetic;[10] at the clinicallly effective concentration, methoxyflurane reduced the postsynaptic sensitivity to acetylcholine at the frog neuromuscular junction, while fluothyl increased the quantal content.[11] We recall that the experimentally derived oil/water partition coefficient of these agents has hitherto failed to provide a guide to their observed effects. In the present approach, we propose that drug specificity is a consequence of the preferential solubility of that drug in a membrane subregion of compatible solubility parameter. This is an extension of the concept first proposed by Mullins[13] and applied by Miller et al.[14] to link solvent property and biophase of action. The issue of concern at that time was a demonstration that the phase in which narcotic action takes place has a $\delta$ value consistent with a hydrocarbon rather than water or protein.[15] In the present concept, we view the hydrophobic core of the membrane as being heterogenous in terms of cohesive energy density, consisting of specific subregions, which may differ significantly in solubility parameter from each other and from the surrounding bulk lipid. These subregions may house various gating molecules such as the receptors for GABA, glutamate, or acetylcholine and entities involved in transmitter release. The drugs under consideration are expected to "dissolve" in these subregions to an extent which is best anticipated by $\delta$.

A given drug will distribute itself among the exophase (the extracellular fluid), the bulk lipid, and the specific subregion according to the scheme shown in Figure 2 (inset). Here $K_{0/w}$ is the membrane/water partition coefficient, $K_{2/1}$ is the partition coefficient within the membrane, and $K_3$ is the partition coefficient between the exophase and the relevant membrane subregion and is equal to $K_{0/w} \times K_{2/1}$. If one assumes the solubility parameter, $\delta_1$, of the bluk lipid to be 9.3,[16] one can employ the known $\delta$ values of the fluorinated ethers[10] and an equation like that of Srebrenik and Cohen[12] (see legend of Figure 2) to compute the dependence of $K_{2/1}$ on $\delta_2$, the *solubility parameter* characterizing a specific membrane

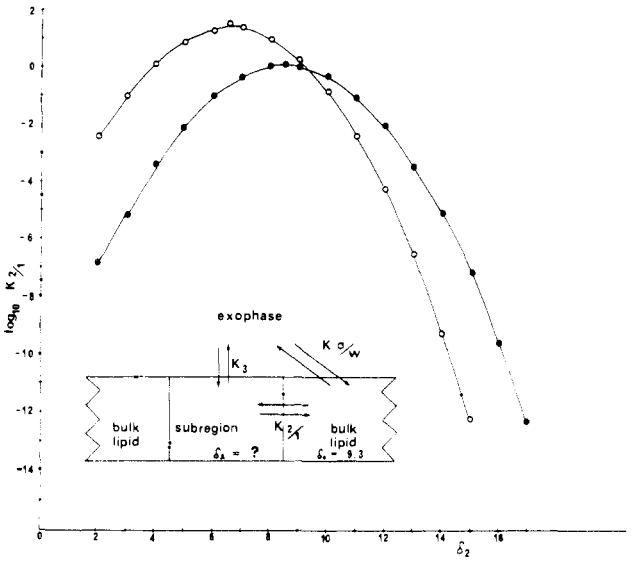


**Figure 2.** The effect of the solubility parameter of a membrane subregion ($\delta_2$) on the distribution coefficient of a drug between it and the bulk lipid of the membrane ($K_{2/1}$). The inset shows the scheme we propose to represent the membrane. For details, see the text. The graph shows the $K_{2/1}$ values for fluothyl (open circles) and methoxyflurane (closed circles) as functions of $\delta_2$. The $K_{2/1}$ values were computed with the following equation:[12]

$$\ln K_{2/1} = \frac{V_s}{RT}(\delta_1 - \delta_s)^2\left(1 + \frac{\delta_1^2}{(\partial E_1/\partial V_1)} - \frac{RT}{(\partial E_1/\partial V_1)V_1}\right) - (\delta_2 - \delta_s)^2\left(1 + \frac{\delta_2^2}{(\partial E_2/\partial V_2)} - \frac{RT}{(\partial E_2/\partial V_2)V_2}\right) - \upsilon_s\left(\frac{1}{V_1} - \frac{1}{V_2}\right)$$

where $V_s$ and $\delta_s$ have the same meaning as in eq 2. For methoxyflurane these were taken as 116 mL and 8.54 $(cal/mL)^{1/2}$ and for fluothyl as 129 mL and 6.59 $(cal/mL)^{1/2}$, respectively.[10] $\delta_1$ of the bulk lipid was taken as 9.3 $(cal/mL)^{1/2}$.[13] $(\partial E_1/\partial V_1)$, the internal pressure of the bulk lipid, was taken as $\delta_1^2$; $(\partial E_2/\partial V_2)$, the corresponding value for the subregion, was taken as $\delta_2^2$. $V_1$ and $V_2$, the molar volomes of phase 1 and 2, were taken as 1000 mL, a value not unreasonable for membrane lipids. The curves do not change significantly when larger values of $V_1$ or $V_2$ are taken.

subregion. The result of such a computation for methoxyflurane and fluothyl is shown in Figure 2 (for details of computation, see figure legend). It is clear that when $\delta_2 > \delta_1$ the subregion will select methoxyflurane over fluothyl and when $\delta_2 < \delta_1$ it will select fluothyl over methoxyflurane. One can employ the curves of Figure 2 to obtain an estimate for the $\delta$ value of a given subregion as follows: We assume that fluothyl and methoxyflurane have an identical action in a given membrane subregion (evidence for this is offered in ref 11) and that $K_{0/w}$ is one-fifth of the experimental octanol/water partition coefficient.[6] We then use the ratio between equipotent concentrations of fluothyl and methoxyflurane to compute the ratio between the $K_{2/1}$ value of the two agents for a given membrane subregion; thus:

$$\frac{K_{2/1}}{K_{2/1}'} = \frac{C_e' K_{0/w}'}{C_e K_{0/w}} \qquad (1)$$

where a prime denotes one of the drugs, say fluothyl, and $C_e$ is the equipotent concentration in the exophase. Knowing $K_{2/1}/K_{2/1}'$ one can directly obtain from Figure 2 the solubility parameter of the membrane subregion related to a given function. A few such estimates based on data from our previous publications[7,11] are given in

Table I. Solubility Parameters of Specific Subregions Involved in Various Membrane Functions as Computed from the Equipotent Concentration Ratios of Methoxyflurane and Fluothyl

| prep | function | drug-induced parameter | ref | $K_{2/1}'/K_{2/1}$; fluothyl/methoxyflurane | $\delta_2$, (cal/cm$^3$)$^{1/2}$ |
|---|---|---|---|---|---|
| frog, neuromusc junct | unknown | 50% return of quantal content toward the control value | 11 | 1/5000 | 13.5 |
| | unknown | 50% of increase in quantal content | 11 | 1/333 | 12.3 |
| | Ach recept | 50% reduct in mepp size | 11 | 1/7 | 10.4 |
| crab, adductor muscle | glutamate recept | 50% reduct of conduct. change caused by glutamate | 7 | 1/23.5 | 11 |
| | GABA recept | 50% reduct of conduct. change caused by GABA | 7 | 100/1 | 6.6 |

Table I. It can be seen that a few membrane subregions, notably those containing the glutamate and the acetylcholine receptors, can be characterized by $\delta_2$ values higher than that of the bulk lipid. On the other hand, the subregion containing the GABA receptor has a $\delta_2$ value much lower than that of the bulk lipid. We do not know whether a given subregion is identical with the boundary lipids[17] or with the hydrophobic core of the relevant membrane proteins, and thus we are unable to attribute to this difference in apparent $\delta$ a concrete physical meaning. Also, the bulk lipid parameters that we used are probably not accurate, as they do not represent "pure" bulk lipid but rather an average of the different membrane components, as $\delta_{\text{apparent}} = \sum \Phi_i \delta_i$. However, the qualitative picture that emerges from Table I, i.e., that $\delta$ of the microenvironment of the GABA receptor is lower than that of the bulk lipid while that of the glutamate receptor is higher, is probably correct.

Regular solution theory enables one to predict the concentration, $X$, of a solute, s, in a given solvent, m, relative to the concentration it would have in an ideal solution at the same chemical potential ($X_I$):

$$\frac{X}{X_I} = \exp\left(-\frac{V_s\Phi_s^2(\delta_s - \delta_m)^2}{RT}\right) \qquad (2)$$

where $V_s$ is the molal volume of s, $\Phi_s$ its volume fraction, and $\delta_s$ and $\delta_m$ are the solubility parameters of the solute and solvent, respectively. The use of eq 2 or its more refined version[12] given under Figure 2 to describe the dissolution of drugs in the membrane is justified only as a first approximation. We do not know how effective this approach will be when applied to drugs outside our homologous series. However, it should be mentioned that mutual solubility computation involving $\delta$ is useful even in situations which clearly do not fulfill the basic assumptions of the regular solution theory[9,12] and can perhaps be employed as long as the geometric mean assumption is not extremely violated, as in cases where a dipole is at the core of a molecular structure rather than at the periphery. The breakdown of this assumption may explain why the $K_{0/w}$ values of the fluorinated ethers cannot be predicted from the $\delta$ values. The use of this theory leads to the suggestion that the gating molecules in the membrane are localized within subregions which are characterized by distinct *solubility parameters*. This may explain the specific action of drugs which do not exhibit clear steric or structural interactions with the membrane.

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