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EFFECTS OF FREE FATTY ACIDS, ETHANOL AND DEVELOPMENT ON γ-AMINOBUTYRIC ACID AND GLUTAMATE FLUXES IN RAT NERVE ENDINGS*

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Abstract—The effects of type A (*cis*-unsaturated) and type B (*trans*-unsaturated and saturated) fatty acids, 1% and 3% ethanol (v/v), and development (7 days) on the thermodynamics of glutamate and γ-aminobutyric acid (GABA) transport into cortical rat brain nerve endings were examined. The effects of the various manipulations, which are known to affect membrane fluidity, may be summarized. Three percent ethanol and oleic acid increased ΔS° and $\Delta S^\dagger$ for glutamate transport and decreased ΔH° and $\Delta H^\dagger$. Type B fatty acids had the opposite effects. In comparison to glutamate transport, GABA transport was less affected by the various manipulations and showed less specificity in terms of the fatty acid effects. Similarly, the effects of development on the thermodynamic parameters for glutamate and GABA transport were not consistent. Glutamate transport into 7-day nerve endings showed thermodynamic behavior similar to that seen when type A fatty acids were incorporated into adult nerve endings. In contrast, GABA transport into 7-day nerve endings had the character of adult nerve endings into which type B fatty acids were incorporated.

The importance of the physiochemical state of biological membranes in regulating functional activities is now widely recognized [1–4]. Understanding the relationships between membrane structure and function has a special place in neuropharmacology since a variety of drugs including the alcohols and gaseous anesthetics are thought to act by perturbing membrane structure [5–8]. However, the precise mechanisms involved are unclear and controversial [9, 10].

Recent studies suggest that a variety of membrane perturbants, including ethanol, increase synaptic membrane "fluidity" [6, 11–14]. (Fluidity is used here to describe a general membrane property which includes both the viscosity of the membrane lipids and the mobility of the membrane proteins within the lipid matrix.) Surprisingly though, there have been few studies (e.g. Ref. 14) which have compared the functional effects of different fluidizing perturbants to see if they produce equivalent effects as would be predicted from the physiochemical measurements. In the present study, we have compared the effects of ethanol and free fatty acids on glutamate and γ-aminobutyric acid (GABA) transport.

The incorporation of free fatty acids into membranes is a technique which has been widely used to study the relationships between membrane fluidity and function [15–22]. This technique seems particularly well suited to study brain membrane structure–function relationships since the free fatty acids can be inserted into synaptic membranes under conditions which maintain synaptic viability. Previous studies have shown that *cis*-unsaturated fatty acids are more effective than *trans*-unsaturated or saturated fatty acids to fluidize membranes and affect function [15, 16]. Klausner *et al.* [16] have presented evidence to suggest that *cis*-unsaturated fatty acids (type A fatty acids) selectively partition into liquid domains of membranes while *trans* and saturated fatty acids (type B fatty acids) selectively partition into gel domains.

In addition to ethanol and free fatty acids, we have also examined GABA and glutamate fluxes in developing (7-day) nerve endings. It is known that 7-day synaptic membranes are more fluid than adult membranes as judged by differences in the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) [23]. The difference in polarization between adult and 7-day membranes compares favorably with the change in the polarization of adult membranes induced by 1% ethanol or the incorporation of 5 mole % of vaccenic acid [14, 23]. Although there are a number of obvious technical problems associated with comparing developing and adult nerve endings, the developing nerve ending (and synaptic membrane) is a unique preparation for studying structural-functional relationships since it is a readily available source of membrane with markedly different physiochemical properties.

MATERIALS AND METHODS

Materials. γ-[^{14}C-(U)]Aminobutyric acid (200 mCi/mmole) γ-[2,3-^{3}H-(N)]aminobutyric acid (35 Ci/mmole) and L-[2,3-^{3}H]glutamic acid (20 Ci/mmole) were purchased from the New England Nuclear Corp. (Boston, MA).

Preparation of adult nerve endings. Nerve endings were prepared from cerebral cortex of adult (180 > age > 60 days) rats exactly as described elsewhere [24]. The morphology and some general chem-

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ical characteristics of these nerve endings have been described [24, 25].

Preparation of 7-day nerve endings. Nerve endings were prepared from 7-day rat cortex as described elsewhere [25]. Only those nerve endings which showed the same buoyant density and morphological appearance as the adult nerve endings were used for this study. These nerve endings sediment between 8 and 12% Ficoll-isotonic sucrose when centrifuged for 45 min at 63,000 *g* (average) on discontinuous Ficoll-sucrose gradient containing successive layers of 6, 8, 12 and 15% Ficoll in 0.32 M sucrose + 5 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), pH 7.8. As judged by morphology [25], enrichment in Na^+,K^+-ATPase activity, and contamination with succinic dehydrogenase (mitochondria), RNA and NADPH cytochrome *c* reductase (endoplasmic reticulum), the purity of the 7-day nerve endings compares favorably with the purity of the adult nerve endings.

Measurement of GABA and glutamate transport. Transport was measured using a dual-labeling procedure [24]. The blanks for all measurements were obtained by incubating samples in the absence of NaCl. Iso-osmolarity was maintained by adding sucrose. The kinetic parameters, K_m and V_{max}, for transport were determined by means of the computer analysis suggested by Wilkinson [26]. The calculation of the thermodynamic parameters from the transport data was performed as described elsewhere [27]. Briefly, the equilibrium thermodynamic parameters were determined as follows: $\Delta G^\circ = RT \ln K_m$ where $R = 1.98$ cal/mole/degree. ΔH° was determined graphically from the plot of $\ln K_m$ vs $1/T$ where the slope = $\Delta H^\circ/R$. ΔS° was determined from $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$. The activation thermodynamic parameters were calculated as follows. $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were determined graphically from the plot of $\ln (V_{max}/T)$ vs $1/T$ where the slope is $-\Delta H^\ddagger/R$ and the intercept is $1\,(R/Nh) + \Delta S^\ddagger/R$ where N = Avogadro's number and h is Planck's constant. $\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$. Unless otherwise noted, ΔG° and $\Delta G^\ddagger$ were determined at 303 K. Statistical analysis of the results was by Student's *t*-test for unpaired data.

Fatty acid insertion. The procedure used was essentially that of Orly and Schramm [15]. The fatty acids were dissolved in absolute ethanol at a concentration of 100 mM. Nerve endings were resuspended in 0.32 M sucrose–5 mM HEPES (pH 7.8) at a concentration of 3 mg protein/ml. The fatty acid was added to the nerve ending suspension to give a final concentration ranging from 0.01 to 1.00 mM. Control nerve endings received ethanol only. The incubations were continued for 30 min at 2–4°C and were terminated by centrifugation (10 min × 40,000 *g*). The pellet was washed once and then resuspended in sucrose–HEPES to a concentration of 0.5 mg protein/ml and used immediately. A portion (3–6 mg protein) was used to prepare synaptic membranes [23]. After harvesting, the membrane lipids were extracted as described elsewhere [23]. A portion of the total lipid extract containing at least 200 nmoles of lipid P was subjected to methanolysis with 1 ml of 12% BCl_3 in a nitrogen atmosphere at 90° for 1 hr. After cooling, 1 ml of distilled water and 2 ml of heptane were added. The methyl esters were extracted into the heptane by shaking for 10 min and then separating the layers with a brief centrifugation; 1.6 ml of the heptane layer was removed, evaporated under vacuum, and resuspended in 20 μl of chloroform. One to three μl of the suspension was subjected to GC analysis on a ¼ in. × 6 ft. column of 10% SP-2330. The chromatograph was temperature programmed from 170 to 210°C at 1°/min with a N_2 flow rate of 30 ml/min.

RESULTS

Effects of temperature on glutamate and GABA transport. The data in Fig. 1 illustrate that from 15 to 37°C the plots of both $\ln K_m$ vs T^{-1} and $\ln (V_{max}/T)$ vs T^{-1} were linear, indicating that both ΔH° (the standard enthalpy) and $\Delta H^\ddagger$ (the activation enthalpy) are constant over this temperature range [27]. A summary of the thermodynamic parameters is given in Table 1. GABA and glutamate transport values were found to have similar ΔG° and ΔH° values; however, ΔS° was significantly higher for GABA transport. Although both GABA and glutamate had similar free energies of activation, $\Delta G^\ddagger$, the relative contributions of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ to $\Delta G^\ddagger$ were somewhat different for the two substrates. $\Delta H^\ddagger$ (a positive term) was significantly greater and

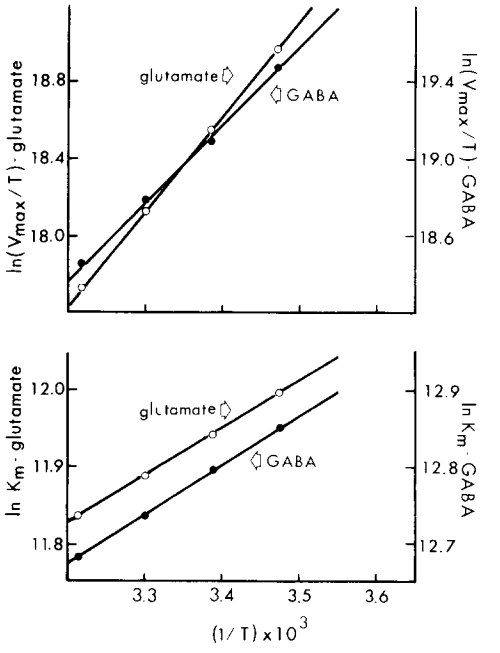

Fig. 1. Plots of $\ln K_m$ vs T^{-1} and $\ln (V_{max}/T)$ vs T^{-1} for GABA and glutamate transport into rat brain nerve endings. GABA and glutamate transport values into rat brain nerve endings were determined simultaneously at 15°, 22.5°, 30° and 37°C. At least six concentrations of substrate were used at each temperature; each experiment was performed in duplicate. Transport blanks were prepared by incubating the samples at the various temperatures in an Na^+-free buffer. K_m and V_{max} values were calculated from the plot of S/V vs S using the computer analysis suggested by Wilkinson [26]. N equals a minimum of twenty-two experiments. The standard deviation of the mean for all points was less than 5%.

$\Delta S^{\dagger}$ (a negative term) was significantly lower for GABA transport as compared to glutamate transport. These data suggest that for glutamate transport a greater ordering of the system occurs before translocation can proceed.

It should be noted that neither the K_m nor the V_{max} values used for these calculations (Table 1) are the simple equilibrium constants or reaction rates assumed by classical thermodynamic analysis. However, the linear nature of the thermodynamic plots suggests that either one constant of several which comprise the observed K_m or V_{max} values is rate-limiting over the temperature range studied or that all the individual constants show essentially a similar temperature dependency [27].

Effects of free fatty acids, ethanol and development on glutamate and GABA transport. The data in Tables 2–5 summarize the effects of the various membrane manipulations on K_m, V_{max} and the thermodynamic parameters for GABA and glutamate transport. The effect of each condition was examined at 15, 22.5 and 30°. Experimental $\Delta H^{\circ}/R$ and $\Delta H^{\dagger}/R$ values were calculated from the slope of the line [ln K_m or ln (V_{max}/T) vs $1/T$] obtained by linear regression analysis. The data have been arranged in decreasing order of the 15°/30°C ratio for K_m (Tables 2 and 4) or for V_{max} (Tables 3 and 5). It is important to note the differences in sign of the various thermodynamic parameters. Thus, for example, an increase in the ΔS° implies the overall reaction is proceeding to a system of greater disorganization, while an increase in $\Delta S^{\dagger}$ implies an increase in the order of the reacting molecules in the transition state before translocation occurs.

Three percent ethanol caused the most significant increase in the glutamate 15°/30°C K_m ratio; this change was reflected by a marked decrease in ΔH° (−120%) and a marked increase in ΔS° (+79%) (Table 2). *cis*-Vaccenic and oleic acids caused similar but more modest changes in these thermodynamic

Table 1. Thermodynamic parameters of glutamate and GABA transport into rat brain*

	ΔG°	ΔH°	ΔS°	$\Delta G^{\dagger}$	$\Delta H^{\dagger}$	$\Delta S^{\dagger}$
	(kcal/mole)		(e.u.)	(kcal/mole)		(e.u.)
Glutamate	−7.03	−3.39	+12.10	+14.37	+6.99	−24.37
	(±0.45)†	(±0.21)	(±0.82)	(±1.34)	(±0.55)	(±1.11)
GABA	−7.89	−2.89	+16.5‡	+14.59	+9.12‡	−18.06‡
	(±0.36)	(±0.11)	(±0.95)	(±1.55)	(±0.67)	(±1.23)

* Experimental details are given in the legend to Fig. 1. $\Delta H^{\circ}/R$ and $\Delta H^{\dagger}/R$ were obtained graphically from the slope of ln K_m vs T^{-1} and ln (V_{max}/T) vs T^{-1} respectively. $\Delta S^{\dagger}$ was obtained graphically from the y-intercept of ln (V_{max}/T) vs T^{-1}. ΔG° was calculated from $\Delta G^{\circ} = RT \ln K_m$ where $T = 30°C$ and ΔS° was obtained from $\Delta S = (\Delta H^{\circ} - \Delta G^{\circ})/T$.

† Numbers in parentheses are the standard errors of the means.

‡ Significantly different from respective glutamate value, $P < 0.05$.

Table 2. Effects of free fatty acids, ethanol and development on glutamate transport: K_m, ΔH° and ΔS° effects

Condition*	Concn	% Incorp.†	Percent change from control				
			K_m-15°C‡	K_m-30°C	K_m-15°/K_m-30°	ΔH°§	ΔS°
Ethanol	3% (v/v)		−15 ± 3‖	−40 ± 6¶	+41 ± 4¶	−120 ± 11¶	+79 ± 8¶
cis-Vaccenic (m.p. = 13°C)	1 mM	12.8	+83 ± 9¶	+39 ± 5¶	+32 ± 4¶	−97 ± 12¶	+83 ± 9¶
Oleic (m.p. = 16°C)	1 mM	11.4	+35 ± 6¶	+21 ± 3¶	+12 ± 3	−42 ± 7¶	+34 ± 2¶
7-Day			+45 ± 7¶	+43 ± 4¶	+1 ± 2	−4 ± 3	−4 ± 1
Palmitic (m.p. = 63°C)	1 mM	14.1	−22 ± 4¶	−11 ± 3	−13 ± 3	+42 ± 3¶	−39 ± 5¶
Ethanol	1% (v/v)		−9 ± 4	+10 ± 4	−18 ± 4¶	+62 ± 7¶	−57 ± 6¶
Stearic (m.p. = 69°C)	1 mM	7.8	−13 ± 3	+6 ± 2	−18 ± 2¶	+66 ± 4¶	−63 ± 6¶
trans-Vaccenic (m.p. = 44°C)	1 mM	9.8	−20 ± 3¶	+8 ± 2	−26 ± 3¶	+101 ± 13¶	−95 ± 8¶

* Nerve endings were either preincubated with 1 mM fatty acid per 3 mg protein per ml as described in Materials and Methods or 1% or 3% (v/v) ethanol was added to the final incubation mixture.

† Percent incorporated refers to the percentage of total membrane fatty acid that is *cis*-vaccenic, oleic, etc.

‡ K_m and V_{max} values were calculated for each condition at 15°, 22.5° and 30°C (22.5°C data not shown).

§ The thermodynamic parameters were determined as described in the legends to Fig. 1 and Table 1. ΔH° is a negative term (see Table 1) while ΔS° is a positive term.

‖ All data are the mean percent changes from control ± S.E.; N = six experiments/group.

¶ Significantly different from control, $P < 0.05$.

Table 3. Effects of free fatty acids, ethanol and development on glutamate transport: V_{max}, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ effects*

Condition	Percent change from control				
	V_{max}-15°C	V_{max}-30°C	V_{max}-15°/V_{max}-30°	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
7-Day	−6 ± 3	−25 ± 4†	+26 ± 4†	−39 ± 5†	+38 ± 4†
Ethanol-3% (v/v)	−20 ± 3†	−37 ± 5†	+26 ± 2†	−39 ± 5†	+39 ± 6†
Oleic acid	−58 ± 6†	−66 ± 9†	+23 ± 3†	−35 ± 3†	+41 ± 6†
cis-Vaccenic acid	−39 ± 4†	−43 ± 6†	+7 ± 3	−9 ± 3	+13 ± 4
Stearic acid	−13 ± 4	−2 ± 2	−12 ± 3	+19 ± 5	−20 ± 3†
Palmitic acid	−31 ± 4†	−20 ± 3	−14 ± 4	+26 ± 3†	−24 ± 4†
Ethanol-1% (v/v)	−12 ± 3	+4 ± 2	−17 ± 4	+28 ± 2†	−28 ± 2†
trans-Vaccenic	−57 ± 6†	−48 ± 5†	−18 ± 3†	+33 ± 4†	−27 ± 3†

* Experimental details are given in the legends to Fig. 1 and Tables 1 and 2. $\Delta H^{\ddagger}$ is a positive number while $\Delta S^{\ddagger}$ is a negative number.
† Significantly different from control, $P < 0.05$.

Table 4. Effects of free fatty acids, ethanol and development on GABA transport: K_m, $\Delta H°$ and $\Delta S°$ effects*

Condition	Percent change from control				
	K_m-15°C	K_m-30°C	K_m-15°/K_m-30°	$\Delta H°$	$\Delta S°$
Ethanol-3% (v/v)	−22 ± 3†	−33 ± 4†	+31 ± 4†	−54 ± 6†	+36 ± 4†
cis-Vaccenic acid	+22 ± 3†	+24 ± 3†	−1	+14 ± 4	+11 ± 3
trans-Vaccenic acid	+4 ± 3	+3 ± 3	+1	+4 ± 2	−3 ± 2
Oleic acid	+37 ± 4†	+40 ± 5†	−3	+18 ± 5	−15 ± 3
Ethanol-1% (v/v)	−24 ± 4†	−17 ± 4	−9 ± 3	+47 ± 5†	−25 ± 6
Palmitic acid	−23 ± 4†	−12 ± 3	−12 ± 4	+63 ± 7†	−35 ± 3†
Stearic acid	−4 ± 2	+10 ± 3	−13 ± 4	+65 ± 8†	−36 ± 2†
7-Day	−47 ± 4†	−22 ± 3†	−32 ± 4†	+166 ± 12†	−93 ± 10†

* Experimental details are given in the legends to Fig. 1 and Tables 1 and 2.
† Significantly different from control, $P < 0.05$.

Table 5. Effects of free fatty acids, ethanol and development on GABA transport: V_{max}, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ effects*

Condition	Percent change from control				
	V_{max}-15°C	V_{max}-30°C	V_{max}-15°/V_{max}-30°	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
Oleic acid	−34 ± 4†	−50 ± 6†	+32 ± 3†	−28 ± 3†	+55 ± 5†
cis-Vaccenic acid	−18 ± 4	−33 ± 4†	+22 ± 2†	−18 ± 4†	+35 ± 4†
Ethanol-3% (v/v)	−30 ± 4†	−41 + 5†	+18 ± 4†	−15 ± 3†	+30 ± 3†
Ethanol-1% (v/v)	+1 ± 1	−6 ± 3	+7 ± 3	−1 ± 1	+3 ± 2
trans-Vaccenic acid	−27 ± 3†	−25 ± 5	+3 ± 1	+11 ± 4	+15 ± 4
7-Day	−28 ± 4†	−21 ± 3†	−10 ± 3	+21 ± 4†	−22 ± 5†
Stearic acid	−12 ± 2	+2 ± 2	−14 ± 3	+26 ± 3†	−35 ± 3†
Palmitic acid	−26 ± 4†	+2 ± 2	−25 ± 2†	+49 ± 5†	−82 ± 7†

* Experimental details are given in the legends to Fig. 1 and Tables 1 and 2.
† Significantly different from control, $P < 0.05$.

parameters. However, the fatty acids and 3% ethanol differed in that the former significantly increased the glutamate K_m, while the latter decreased the K_m. Although the glutamate K_m in 7-day animals was increased significantly at all temperatures, there was no significant change in the thermodynamic parameters. The type B fatty acids and 1% ethanol decreased the 15°/30°C K_m ratio; this change was reflected by an increase in $\Delta H°$ and a decrease in $\Delta S°$. *trans*-Vaccenic acid was the most potent in this regard increasing $\Delta H°$ 101% and decreasing $\Delta S°$ 95%.

The data in Table 2 also show that, when nerve endings (3 mg/ml) were incubated with 1 mM fatty acid, the magnitude of the insertion was such that approximately 10% of the membrane fatty acid was *cis*-vaccenic, *trans*-vaccenic, etc. The levels of incorporation varied from 7.8% for stearic to 14.1% for palmitic. Increasing the *cis*-vaccenic acid concentration from 0.03 to 0.1 to 1.0 mM resulted in a progressive increase in incorporation from 0.1 to 3.4 to 12.8%.

The data in Table 3 shows that $\Delta H^{\dagger}$ was decreased significantly and $\Delta S^{\dagger}$ increased in the 7-day preparation. Oleic acid and 3% ethanol produced similar effects. Although *cis*-vaccenic acid significantly decreased the V_{max} values at all temperatures, as did 3% ethanol and oleic acid, *cis*-vaccenic acid had no significant effect on the thermodynamic parameters, $\Delta H^{\dagger}$ and $\Delta S^{\dagger}$. The type B fatty acids and 1% ethanol modestly decreased the 15°/30°C V_{max} ratio and similarly caused a modest increase in $\Delta H^{\dagger}$ and decrease in $\Delta S^{\dagger}$. It is interesting to note that, while *trans*-vaccenic acid was as potent as oleic and *cis*-vaccenic acids in decreasing the V_{max}, the thermodynamic interpretation of the *trans*-vaccenic acid effect was opposite to that of the *cis*-unsaturated fatty acids.

In contrast to the effects on the glutamate K_m, only 3% ethanol significantly increased the GABA 15°/30°C K_m ratio (Table 4) which was reflected in a significant increase in $\Delta S°$ and a decrease in $\Delta H°$. Although *cis*-vaccenic and oleic acids had significant effects on the GABA K_m, these effects were temperature insensitive and thus caused no significant change in the thermodynamic parameters. Similarly, *trans*-vaccenic acid had no significant effect on $\Delta H°$ and $\Delta S°$. Nerve endings treated with stearic and palmitic acids and 1% ethanol and nerve endings from 7-day animals all showed significant increases in $\Delta H°$ and decreases in $\Delta S°$. The 7-day nerve endings were the most affected in this regard showing a 166% increase in $\Delta H°$ and a 93% decrease in $\Delta S°$.

Oleic acid, *cis*-vaccenic acid and 3% ethanol all significantly decreased the GABA V_{max}, an effect which increased with increasing temperature (Table 5). $\Delta H^{\dagger}$ was modestly decreased and $\Delta S^{\dagger}$ was modestly increased in these groups. One percent ethanol and *trans*-vaccenic acid had no significant effect on the thermodynamic parameters. Stearic and palmitic acids significantly increased $\Delta H^{\dagger}$ and decreased $\Delta S^{\dagger}$.

A comparison of the data in Tables 2–5 suggests certain conclusions; (a) 3% ethanol had relatively similar effects on glutamate and GABA transport in terms of temperature-dependent changes in K_m and V_{max} and the thermodynamic parameters, (b) 1% and 3% ethanol generally had different effects on the thermodynamic parameters, (c) for glutamate transport the incorporation of type A and type B fatty acids generally led to opposite effects on the thermodynamic parameters, (d) in contrast, there was less specificity for the fatty acid effect on GABA transport, and (e) the effects of development on the thermodynamic parameters were not consistent between GABA and glutamate transport, e.g. the temperature-dependent changes in the 7-day glutamate V_{max} were similar to those seen after the incorporation of *cis*-unsaturated fatty acids into adult nerve endings, while the effect of development on the GABA K_m was similar to that seen after the incorporation of the saturated fatty acids.

DISCUSSION

The results of the present study illustrate that the measurement of thermodynamic changes in transport processes is an accurate and sensitive technique for assessing the membrane pertubations induced by alcohol and other agents which are known to produce changes in membrane "fluidity". As shown in Tables 2–5, small temperature-dependent changes in K_m and V_{max}, which of themselves may not be significant, can cause significant changes in the equilibrium or thermodynamic activation parameters. Furthermore, the thermodynamic approach excludes interference by non-specific drug effects. For example, both *cis*- and *trans*-vaccenic acid significantly decreased the V_{max} for glutamate transport at all temperatures tested. However, the inhibition by *cis*-vaccenic acid was temperature independent, while the effects of *trans*-vaccenic acid diminished with increasing temperature. Thus, *trans*-vaccenic acid had a significant effect on the activation thermodynamics while *cis*-vaccenic acid had no effect.

Interpretation of the thermodynamic data is not without pitfalls. For example, all of the manipulations tested may cause changes in synaptic viability that could produce apparent thermodynamic changes in K_m and V_{max}. Such changes could include increased synaptosomal leakiness and the consequent changes in ionic gradients, decreased Na^+,K^+-ATPase activity and decreased ATP content. In regard to changing ATP concentrations, Corps *et al.* [28] have found that the inhibition of anti-immunoglobulin cap formation on lymphocytes by *cis*-unsaturated fatty acids is related to the ability of those compounds to penetrate through the cell membrane and uncouple oxidative phosphorylation. Saturated and *trans*-unsaturated fatty acids, which also uncouple oxidative phosphorylation but do not penetrate well into the cell interior, have no effect on cap formation. At least one line of evidence strongly suggests that these phenomena do not play a significant role in our interpretation in the present experiments. General effects on synaptic viability would be expected to have relatively similar effects on GABA and glutamate transport given the similarities in the transport mechanisms [29]. However, the data clearly indicate that the various membrane manipulations have selective effects on glutamate and GABA transport. For example, neither *cis*- nor *trans*-vaccenic acid had significant effects on the equilibrium thermodynamics of GABA transport while both compounds significantly and differently affected the equilibrium thermodynamics of glutamate transport (Table 2).

A second problem in data interpretation involves the observation that homologous manipulations, which are known to produce similar changes in the physical properties of membranes, produced distinctive changes in the transport thermodynamics. For example, the insertion of *cis*-vaccenic and oleic acids did not produce qualitatively similar changes in the activation thermodynamics for glutamate transport. There are, however, other examples (see

Tables 2 and 5) where these fatty acids do cause equivalent effects. Overall, these data probably confirm the earlier observation by Orly and Schramm [15] that the physical properties of the fatty acid are not the sole determinant of effect but that specific molecular structure also plays an important role.

The data also indicate that it is not always possible to generalize or predict effects among heterogeneous manipulations which have some common effects on membrane structure. For example, the data in Table 2 illustrate that the addition of 3% ethanol or the insertion of *cis*-unsaturated fatty acids produced similar effects on the equilibrium thermodynamics, namely a decrease in enthalpy ($\Delta H°$) and an increase in entropy ($\Delta S°$). Restated, these manipulations attenuated or reversed the temperature-dependent increases in the glutamate K_m. However, this phenomenon could not be generalized to the 7-day nerve endings. Although the synaptic membranes in the preparation are significantly more fluid than their adult counterpart [23], the temperature-dependent changes in the glutamate K_m and consequently the equilibrium thermodynamics remained intact.

The data in Tables 2 and 3 also show that oleic acid and 3% ethanol not only increased the spontaneity of glutamate transport (higher $\Delta S°$) but also significantly decreased the enthalpy of activation $\Delta H^{\ddagger}$. These compounds also significantly increased $\Delta S^{\ddagger}$ suggesting that the reacting components, glutamate, the carrier, Na^+ and the membrane, had a greater requirement to orient themselves properly in three-dimensional space and to achieve an appropriate spatial proximity before translocation could occur. In contrast, the type A fatty acids, which may have a net membrane stabilizing effect [16], decreased $\Delta S^{\ddagger}$. It is interesting to note that for glutamate transport, 1 and 3% ethanol did not have a dose-related effect on the activation or equilibrium thermodynamics. In fact, these two concentrations of ethanol produced essentially opposite effects in some paradigms. These data suggest that for ethanol specifically, and perhaps other membrane perturbations generally, it may not be possible to use high drug concentrations to amplify mechanisms occurring at lower pharmacological concentrations.

Overall, the results presented here illustrate the complexities encountered when attempting to evaluate the effects of membrane perturbants on function and when attempting to use such data to develop a consistent hypothesis regarding membrane structure and function. The data clearly illustrate that interpretations for one system may not necessarily apply to another. Finally, the results of the present study illustrate that the various perturbants had marked effects on the kinetic parameters, K_m and V_{max}, some of which appeared to be independent of the related thermodynamic changes. The possibility should be considered that the various perturbants may have selectively lysed or functionally destroyed certain nerve endings and that the results we measured are those of a surviving unique population of particles.

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