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Thermodynamic parameters for [D-Pen^{2,5}]enkephalin at δ-opioid receptors in the mouse isolated vas deferens

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Dissociation constants (K_A) for [D-Pen^{2,5}]enkephalin (DPDPE) inhibition of the electrically evoked twitch of the mouse isolated vas deferens preparation (MVD) were calculated at five temperatures (25, 30, 34, 37 and 40°C). These values were determined from the equiactive concentrations obtained before (A) and after (A') partial irreversible blockade of a fraction of the receptor population by β-chlornaltrexamine (β-CNA) plotted as (1/A) against (1/A') where K_A = (slope − 1)/intercept. The values of K_A tended to increase (approximately doubled) over this temperature range, indicating that the affinity of DPDPE for the opioid δ receptor is an inverse function of temperature. From these results, thermodynamic parameters were calculated from a Van 't Hoff plot of ln(K_A) against 1/T. The relative magnitudes of the change in enthalpy ($\Delta H^{\circ\prime} = -6.67$ kcal mol^{-1}), the change in entropy ($\Delta S^{\circ\prime} = +0.009$ kcal mol^{-1} °K^{-1}) and the change in free energy ($\Delta G^{\circ\prime} = -9.43$ kcal mol^{-1}) suggest that the interaction between DPDPE and the δ-opioid receptor in MVD is an exothermic exergonic reaction, predominantly enthalpy-driven and results in an increase in the entropy of the system.

Thermodynamics; δ-Opioid receptors; Mouse vas deferens; DPDPE ([D-Pen^2,D-Pen^5]enkephalin)

1. Introduction

Thermodynamic analysis has been applied to a variety of ligand-receptor interactions in order to gain information about the fundamental molecular events underlying these interactions not obtainable by other techniques (for review, see Raffa and Porreca, 1989). Implicit in thermodynamic analysis of pharmacologic data is the quantitative measurement of the driving forces involved in the drug-receptor interaction. Such measurement is traditionally incorporated into more global measures such as 'affinity', but thermodynamic analysis offers potential insight into the underlying mechanisms occurring at the receptor level beyond the resolving power of, for example, the dissociation constant. The temperature dependence of the dissociation constant allows the determination of thermodynamic parameters using a Van 't Hoff plot. The introduction of β-chlornaltrexamine (β-CNA) (Caruso et al., 1979; Portoghese et al., 1980) makes the estimation of dissociation constants of opioid agonists in isolated tissue preparations possible by the method of partial receptor inactivation of opioid receptors. β-CNA produces non-competitive (and apparently irreversible) antagonism of opioid receptors in bioassays (Ward et al., 1982) and has been used for estimating opioid receptor dissociation constants in the guinea-pig isolated ileum and mouse vas deferens (MVD) (Porreca and Burks, 1983; Chavkin and Goldstein, 1984; Porreca et al., 1990). The use of thermodynamic analysis in isolated tissue preparations has previously been used for the α_1-adrenoceptor of rabbit thoracic aorta (Raffa et al., 1985).

We recently calculated the thermodynamic parameters for the binding of the antagonist naloxone to δ-opioid receptors in the MVD (Raffa et al., 1992). The purpose of the present study was to calculate and compare the parameters using the highly selective δ receptor agonist [D-Pen^{2,5}]enkephalin (DPDPE) (Mosberg et al., 1983) in the same preparation.

2. Materials and methods

2.1. Mouse isolated vas deferens preparation

Male ICR mice (20–35 g, Harlan) were killed by cervical dislocation and the vasa deferentia were removed. Tissues (4–6 per temperature) were mounted

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between platinum strip electrodes in 20 ml organ baths at a tension of 1 g and bathed in oxygenated (95:5% O_2:CO_2) magnesium-free modified Kreb's buffer at 25, 30, 34, 37 or 40°C. Each was stimulated electrically (0.1 Hz, single pulses, 2.0 ms duration) at the minimal voltage required to produce a maximal tissue response (50–60 V).

2.2. DPDPE administration

DPDPE was added to the tissue baths, one concentration at a time with washing of the tissues between individual applications. Non-cumulative concentration-effect curves were constructed to avoid possible tolerance of the tissue and to prevent possible peptide breakdown during the course of the experiment. DPDPE concentration-effect curves were produced both before and after incubating the tissues with β-CNA.

2.3. β-CNA incubation details

Tissues were incubated at the test temperature with various concentrations of β-CNA (0.5–4.0 μM) (Portoghese et al., 1980) for 30 min. Incubations were followed by 20 washes of the tissue with the modified Kreb's solution followed by three washes every 10 min for the next 50 min. Subsequent to this procedure,

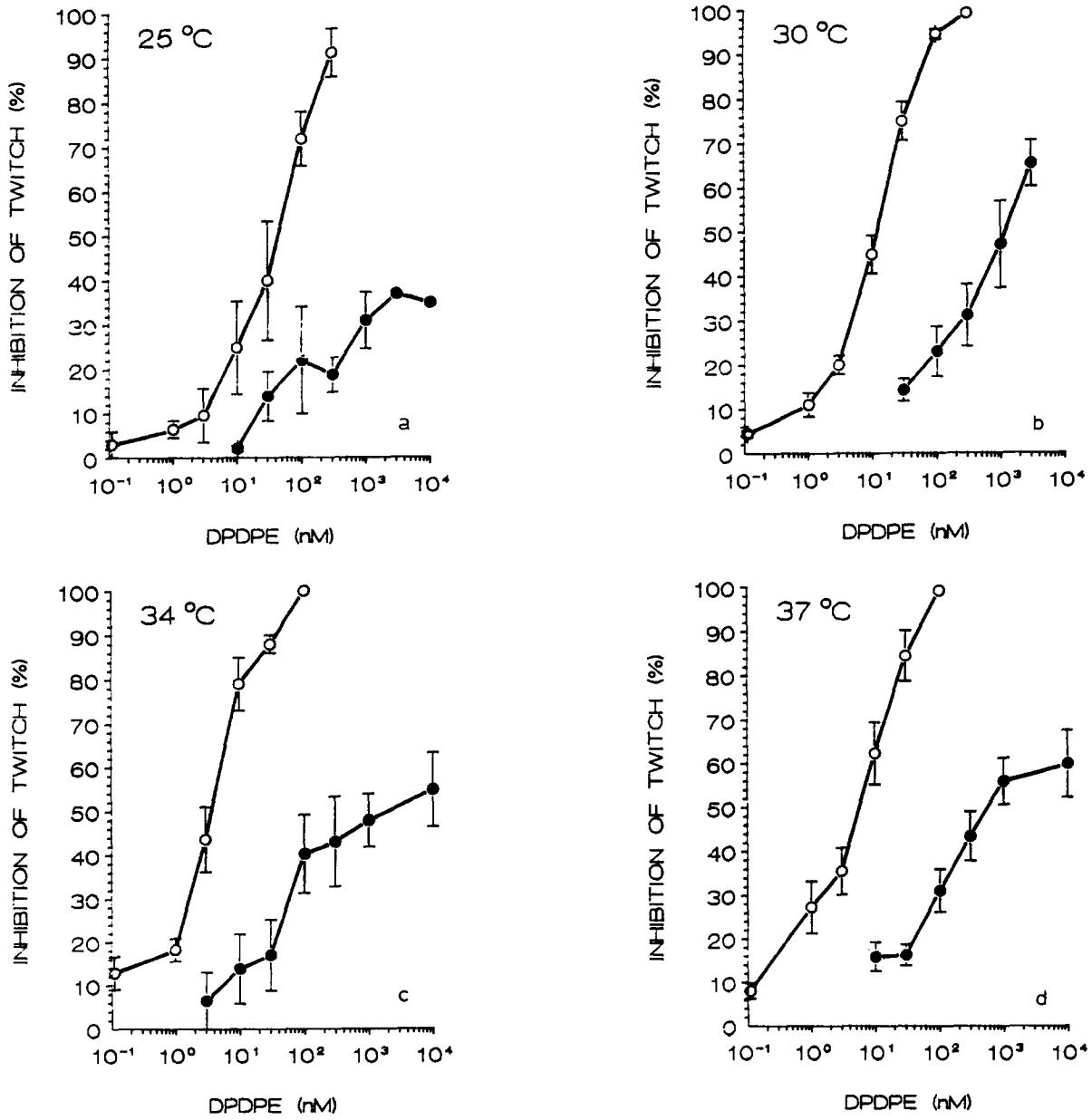

Fig. 1. Concentration-response curves for DPDPE inhibition of electrically evoked contractions of the mouse isolated vas deferens in the absence (open symbol) or presence (closed symbol) of β-CNA at 25, 30, 34, 37 and 40°C. Error is ±S.E.M.

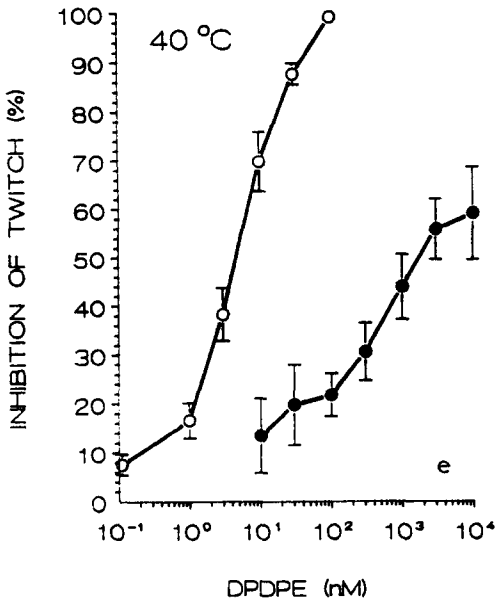

Fig. 1 (continued).

DPDPE concentration-effect curves were again constructed.

2.4. Calculations

The relationship between change in free energy ($\Delta G°'$), change in enthalpy ($\Delta H°'$), and change in entropy ($\Delta S°'$) is given by the equation $\Delta G° = \Delta H° - T\Delta S°$, which applies to standard-state conditions (usually 25°C, 1 atm all components at unit activity and $[H^+] = 1.0$ M). Thermodynamic quantities determined at non-standard-state conditions are represented without superscripts and when determined at other than pH = 0.0, by their primed counterparts. The free energy change for the presumed bimolecular agonist[A]-receptor[R] reaction is given by, $\Delta G = \Delta G° + RT \ln([AR]/[A][R])$, where R is the gas constant (1.99 cal mol^{-1} $°K^{-1}$ = 8.31 J mol^{-1} $°K^{-1}$).

In these experiments, the interaction was allowed to come to equilibrium, at which point $\Delta G = 0$ and the expression [A][R]/[AR] is the dissociation constant K_A. Thus $\Delta G° = RT \ln(K_A)$.

2.5. Dissociation constants

Values of IC_{50} (dose of DPDPE which inhibited twitch by 50%) were determined by fitting curves to the mean DPDPE concentration-effect data (means from minimum of six tissues from individual animals per curve) with the aid of a computer program (Tallarida and Murray, 1986). Values of K_A were obtained using the method of partial irreversible blockade (Furchgott, 1966) in which equiactive concentrations of agonist before (A) and after (A') blockade by an irreversible antagonist are determined and plotted as reciprocals. The resulting plot should be linear, as seen from the equation $(1/A) = (1/q)(1/A) + (1/q - 1)/K_A$, where q is the fraction of receptors unoccupied after irreversible blockade. The value of K_A for DPDPE was calculated at each temperature from the slope and y intercept of this line according to the equation K_A = (slope − 1)/intercept.

The thermodynamic quantities $\Delta G°'$, $\Delta H°'$ and $\Delta S°'$ were calculated in a manner similar to that used previously (Raffa et al., 1985) and by Weiland et al. (1979). Substitution of the expression relating changes in free energy to $\Delta H°'$ and $\Delta S°'$ ($\Delta G°' = \Delta H°' - T\Delta S°'$) into the equation $\Delta G°' = RT \ln(K_A)$ yields the Van 't Hoff equation $\ln(K_A) = (\Delta H°'/R)(1/T) - \Delta S°'/R$. A plot of $\ln(K_A)$ against (1/T) yields a theoretically straight line of slope $\Delta H°'/R$ and y intercept $-\Delta S°'/R$. Because R is known, both $\Delta H°'$ and $\Delta S°'$ can be obtained from such a plot.

3. Results

3.1. Effect of temperature on sensitivity to DPDPE

The maximum inhibition of twitch height produced by DPDPE was close to 100% at each of the temperatures examined (25, 30, 34, 37 and 40°C). The IC_{50} value did not significantly vary with temperature, indicating tissue viability at all of the temperatures.

3.2. Antagonism by β-CNA

After incubation of the tissues with β-CNA, followed by its washout, the maximum inhibitory response to DPDPE was significantly attenuated at each temperature over the range of 25–40°C and in each case the pretreatment with β-CNA produced a rightward and downward shift of the DPDPE concentration-effect curve, characteristic of partial irreversible blockade of a fraction of the total receptor population. Compared to pre-blockade response, the maximal inhibition after β-CNA treatment was reduced to 34, 67, 52, 58, and 56%, respectively.

3.3. Dissociation constants

Dissociation constants were calculated from the concentration-effect curves of DPDPE in the absence and presence of partial irreversible receptor blockade by β-CNA (fig. 1). Pairs of equiactive concentrations in tissues prior to, and after, treatment with β-CNA were plotted as reciprocals (1/A vs. 1/A', respectively) (fig. 2). The double reciprocal plots of these equiactive concentrations were linear (r = 0.971–0.999) for each temperature tested and values of K_A were calculated at

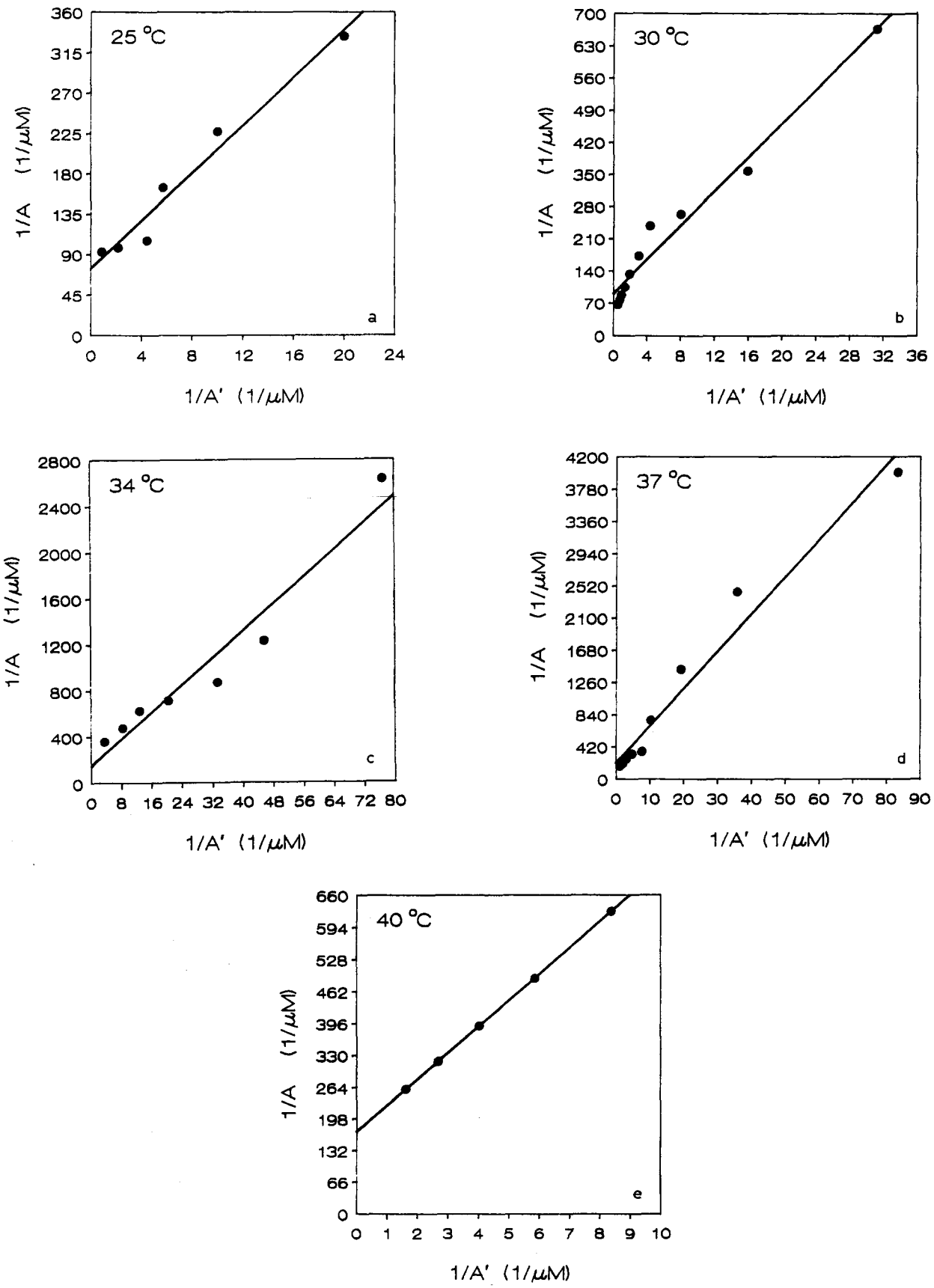

Fig. 2. Double-reciprocal plots of equieffective DPDPE concentrations at each temperature determined from fig. 1.

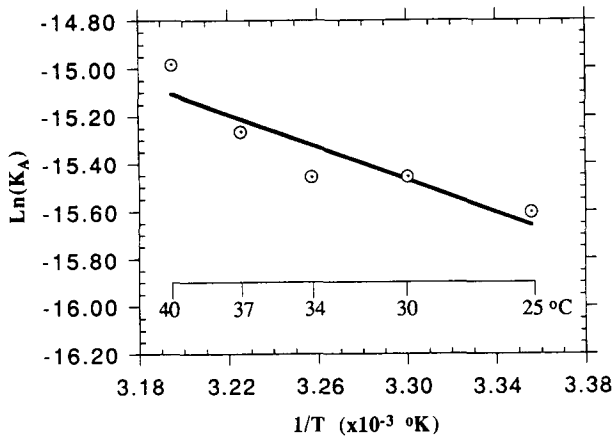

Fig. 3. The Van 't Hoff plot for the interaction of DPDPE with the δ-opioid receptor in the mouse isolated vas deferens determined at five temperatures.

each temperature from the slope and y intercept of these double-reciprocal plots as described in Materials and methods.

3.4. Thermodynamic quantities

Values of K_A for DPDPE obtained at each temperature between 25 and 40°C were used to construct a Van 't Hoff plot of $\ln(K_A)$ against $1/T$, where T is degrees Kelvin (fig. 3). The points were fitted by linear regression analysis with the aid of a computer program (Tallarida and Murray, 1986). From the resulting line, the enthalpy change was calculated from $\Delta H^{\circ\prime} = R \times$ slope and the entropy change was calculated from $\Delta S^{\circ\prime} = -R \times y$ intercept. The change in free energy was calculated in two ways, as $RT \ln(K_A)$ and as $\Delta H^{\circ\prime} - T\Delta S^{\circ\prime}$. There was no significant difference ($P > 0.05$) in the mean values of $\Delta G^{\circ\prime}$ obtained by either method ($\Delta G^{\circ\prime} = -9.35 \pm 0.08$ (S.D.) kcal mol^{-1} and -9.43 ± 0.05 (S.D.) kcal mol^{-1}, respectively). The K_A values and thermodynamic quantities obtained at each temperature are shown in table 1.

4. Discussion

The temperature dependence of the dissociation constant of opioid ligands has been determined in radioligand binding techniques by, for example, Nicolas et al. (1982) for human β-endorphin, Hitzemann et al. (1985) for the interaction of etorphine with membranes prepared from adult Sprague-Dawley rats and Borea et al. (1988) for the binding of radiolabelled [D-Ala^2,N-MPhe^4,Gly-ol^5]enkephalin (DAMGO, μ ligand), [D-Ala^2,D-Leu^5]enkephalin (DADLE, δ ligand) and ethylketocyclazocine (κ ligand) in membranes prepared from guinea-pig brain. Similar approaches have been used extensively in studies of the adrenergic system (e.g., Weiland et al., 1979, 1980; Weiland and Molinoff, 1981). In theory, the thermodynamic parameters of the interaction of a drug with its receptor should also be obtainable using assays in which a pharmacologic response is measured. The only prior report using this approach (Raffa et al., 1985) found close agreement between thermodynamic quantities obtained for norepinephrine-induced contraction of rabbit isolated aortic strips and published data from studies using radioligand binding techniques. Thus, it seems reasonable to expect that the thermodynamics of the interaction between ligands and opioid receptors in appropriate isolated tissue preparations would be amenable to this type of analysis. We report here the study of the temperature dependence of the interaction of the highly selective δ-opioid agonist DPDPE (Mosberg et al., 1983) with the receptor in the mouse isolated vas deferens, a preparation which has previously been used to estimate the affinity of opioid agents (Porreca et al., 1990) at one temperature (37°C).

At all temperatures examined, β-CNA produced a rightward and downward shift of the dose-response curve of DPDPE, characteristic of irreversible blockade of a portion of the DPDPE-sensitive (presumably δ) receptor population. Hence, the dissociation constant could be calculated based on the shifts in the DPDPE dose-response curve at each temperature. This procedure for calculating K_A further eliminates any untoward effect of temperature on tissue mechanics or responsivity. At each temperature examined, the double reciprocal plot ($1/A$ against $1/A'$) was linear with correlation coefficient (Pearson r values) of 0.971–0.999. Thus, the dissociation constants could be calculated from these data, as (slope − 1)/y intercept.

That the observed temperature-dependent changes in K_A could be due to temperature-dependent changes in tissue characteristics does not seem likely. The mechanical integrity of the isolated tissue preparation and response to DPDPE (inhibition of the electrically evoked twitch) was stable over the temperature range

TABLE 1

Thermodynamic parameters for the interaction between DPDPE and the δ-opioid receptor in the MVD over the temperature range 25–40°C.

	25°C	30°C	34°C	37°C	40°C
K_A (nM)	167.0	194.0	194.1	234.8	310.9
$\Delta G^{\circ\prime}$ (kcal/mol) [a]	−9.25	−9.32	−9.44	−9.42	−9.33
$\Delta G^{\circ\prime}$ (kcal/mol) [b]	−9.35	−9.40	−9.43	−9.46	−9.49
Mean $\Delta G^{\circ\prime}$ (kcal/mol) [a] = -9.35 ± 0.08					
Mean $\Delta G^{\circ\prime}$ (kcal/mol) [b] = -9.43 ± 0.05					
$\Delta H^{\circ\prime}$ (kcal/mol) [b] = −6.67					
$\Delta S^{\circ\prime}$ (kcal/mol °K) [b] = +0.009					

[a] Calculated from $\Delta G^{\circ\prime} = RT \ln(K_A)$. [b] Calculated from Van 't Hoff plot: $\Delta H^{\circ\prime} = R \times$(slope), $\Delta S^{\circ\prime} = -R \times$(y intercept) and $\Delta G^{\circ\prime} = \Delta H^{\circ\prime} - T\Delta S^{\circ\prime}$.

examined in this study as evidenced by the lack of significant difference in IC_{50} values (1.4–5.0 nM) at any temperature. Although a consistently lower IC_{50} was observed at 30°C, the standard error of the IC_{50} value was also greatest at this temperature. Overall, there was a consistent tissue sensitivity against which the dissociation constant of DPDPE could be measured.

The principal finding of this study is that the dissociation constant (K_A) of DPDPE in mouse isolated vas deferens is a reciprocal function of temperature over the temperature range examined (25–40°C). It appears, therefore, that the affinity of DPDPE for the δ-opioid receptor is inversely related to temperature over this range. Based on the temperature dependence of the K_A of DPDPE, the thermodynamic quantities $\Delta G°'$, $\Delta H°'$, and $\Delta S°'$ were calculated from an analysis of a Van 't Hoff plot of the K_A versus temperature data. Based on this analysis, the drug (DPDPE) receptor (δ-opioid) interaction is exergonic, i.e., occurs with a negative free energy change ($\Delta G°' < 0$), confirming that the reaction occurs spontaneously in response to favorable thermodynamic conditions. Also based on analysis of the Van 't Hoff plot, the interaction occurs with a negative change in enthalpy ($\Delta H°' < 0$) and a slight positive change in entropy ($\Delta S°' > 0$).

There have been a few radioligand binding studies that have investigated the temperature dependence of opioid ligand affinity and the associated thermodynamics of the interaction with ligand binding sites. One of these studies, by Nicolas et al. (1982), found that the binding of β-endorphin to adult male Sprague-Dawley rat brain membranes was markedly temperature-dependent (0–40°C) and occurred with values for the thermodynamic quantities of $\Delta G°' = -12.4$ kcal mol^{-1}, $\Delta H°' = 11.0$ kcal mol^{-1}, and $\Delta S°' = 0.08$ kcal mol^{-1} $°K^{-1}$ in the absence of 100 nM Na^+ and similar values ($\Delta G°' = -11.0$, $\Delta H°' = 15.5$ $kcal^{-1}$ mol^{-1}, and $\Delta S°' = 0.09$ kcal mol^{-1} $°K^{-1}$) in the presence of 100 nM Na^+. Hitzemann et al. (1985) showed that the binding of [^{3}H]etorphine to whole rat brain homogenates occurred with a negative change in free energy ($\Delta G°' = -13.72$ kcal mol^{-1}), a small positive change in enthalpy ($\Delta H°' = 2.31$ kcal mol^{-1}), and a positive change in entropy ($\Delta S°' = 0.052$ kcal mol^{-1} $°K^{-1}$). Borea et al. (1988) examined the thermodynamics of binding of ligands in guinea-pig brain and found that DAMGO, DADLE and ethylketocyclazocine binding occurred with a negative change in $\Delta G°'$ (−11.98 to −12.3 kcal mol^{-1}), a positive change in $\Delta H°'$ (2.38–4.35 kcal mol^{-1}) and a positive change in $\Delta S°'$ (0.048–0.055 kcal mol^{-1} $°K^{-1}$).

The results of the present study, using an isolated tissue preparation, agree quantitatively as well as qualitatively for $\Delta G°'$ and $\Delta S°'$ with the radioligand binding studies cited above. The magnitude of the $\Delta G°'$ measured at each temperature (−9.25 to −9.49 kcal mol^{-1}) is close to the range of $\Delta G°'$ values measured in the binding studies. It is also within the range of values obtained in radioligand binding studies at other receptor types (for review, see Raffa and Porreca, 1989).

The change in enthalpy ($\Delta H°'$) determined in the present study was negative, indicating that the interaction of DPDPE with the receptor population in MVD is exothermic. The change in entropy ($\Delta S°'$) observed in this study was positive, also favoring the reaction in the direction of binding. The magnitude of the $\Delta S°'$ term was sufficient to contribute to the total free energy change. Apparently, the overall reaction between DPDPE and the δ-opioid receptor of MVD occurs spontaneously due to the combined contributions, but the predominant contribution is the increase

TABLE 2

Summary of thermodynamic analyses of opioid ligand interactions.

	Preparation	$\Delta G°'$	$\Delta H°'$	$\Delta S°'$	Reference
Agonists					
(a) Radioligand binding					
β-Endorphin	Rat brain	< 0	> 0	> 0	Nicolas et al. (1982)
Etorphine	Rat brain	< 0	> 0	> 0	Hitzemann et al. (1985)
DAMGO (μ)	Guinea-pig brain	< 0	> 0	> 0	Borea et al. (1988)
DADLE (δ)	Guinea-pig brain	< 0	> 0	> 0	Borea et al. (1988)
Ethylketazocine (κ)	Guinea-pig brain	< 0	> 0	> 0	Borea et al. (1988)
(b) Isolated tissue					
DPDPE	MVD	< 0	< 0	> 0	This study
Antagonists					
(a) Radioligand binding					
Diprenorphine	Rat brain	< 0	< 0	> 0	Hitzemann et al. (1985)
(b) Isolated tissue					
Naloxone	MVD	< 0	< 0	< 0	Raffa et al. (1992)

Key: DAMGO = [D-Ala^2,N-MePhe^4,Gly-ol^5]enkephalin; DADLE = [D-Ala^2,D-Leu^5]enkephalin; DPDPE = [D-Pen^{2,5}]enkephalin; MVD = mouse vas deferens.

in enthalpy, i.e., the interaction is mainly enthalpy-driven. This finding, in an isolated tissue preparation, differs from the radioligand binding studies (table 2). For example, Nicolas et al. (1982) found the binding of human β-endorphin to rat brain membranes to be strongly entropy-driven. Hitzemann et al. (1985) reported that the binding of etorphine (agonist) was entropy-driven and that the binding of diprenorphine (antagonist) occurred in a manner that was both enthalpy- and entropy-favorable. Borea et al. (1988) found the binding of three opioid subtype-selective agonists to be entropy-driven. Hence, in radioligand binding studies, the interaction of opioid agonist appears to be entropy-driven. In both radioligand binding and isolated tissue preparation, the interaction of opioid antagonist appears to be enthalpy-driven. Perhaps the difference is in the lack of a coupled transduction system. That the thermodynamics of interaction may be receptor-specific is shown by the fact that, for example, the β-adrenoceptor agonist binding is enthalpy-driven and antagonist binding is largely entropy-driven (e.g., Weiland et al., 1979, 1980; Bree et al., 1986; Contreras et al., 1986). We recently reported on the thermodynamic analysis of the temperature dependence of the dissociation constant (K_B) of naloxone in this tissue (Raffa et al., 1992). The affinity of naloxone was found to be an inverse function of temperature. The thermodynamic quantities ($\Delta G^{\circ\prime} = -10.59$ kcal/mol, $\Delta H^{\circ\prime} = -15.73$ kcal/mol and $\Delta S^{\circ\prime} = -0.0168$ kcal/mol $^{\circ}K^{-1}$) suggest that the interaction between naloxone and the δ-opioid receptor in the MVD is enthalpy-driven. It is interesting to note that in the same tissue preparation (MVD) the interaction of the opioid antagonist (naloxone) as well as the opioid agonist (DPDPE) were found to be enthalpy-driven. The investigation of the generality of this finding to other opioid agonists and antagonists should be pursued.

Although there are obvious limitations to the measurement of thermodynamic quantities in either isolated tissue preparations or radioligand binding assays, when one compares the quantities obtained by each method for the same receptor type, a striking uniformity emerges. This is true of the present study, as discussed above, and of the only other report of the measurement of thermodynamic quantities using an isolated tissue preparation. Raffa et al. (1985) measured the thermodynamic quantities for the interaction of norepinephrine with the α_1 adrenoceptor of rabbit isolated thoracic aorta. There was a close agreement between thermodynamic quantities determined in the isolated tissue preparation and radioligand binding assays as compared to the greater difference (typically an order of magnitude) between dissociation constants in the two systems. It is intriguing to note that the agreement in thermodynamic quantities is closer than the agreement in other quantities related to the drug-receptor interaction, such as the dissociation constant. The implication is that the thermodynamic measurements reveal a uniformity in the fundamental driving forces of the drug-receptor interaction that are beyond the resolving power of other quantitative measurements.

A finding in the present study is that the interaction between exogenously applied DPDPE and the δ-opioid receptor of MVD occurs with a relatively modest negative change in enthalpy. Speculatively, an endogenous ligand for this receptor might bind in an even more energetically favorable manner. The increase in enthalpy observed in the present study also implies that the interaction between DPDPE and the receptors on MVD occurs only because of the increase in entropy. It has been speculated that an increase in entropy (increased randomness) could be brought about by several factors, including the displacement of ordered water molecules from around the receptor site (see, for example, discussion by Weiland et al., 1979), the breaking of (hydrogen or Van der Waals) bonds leading to the receptor assuming a more open configuration so that there is greater freedom of movement in the unfolded ligand-receptor complex (see, for example, discussion by Hitzemann et al., 1985) or the presence of hydrophobic forces driving the formation of the transition state (see, for example, discussion by Nicolas et al., 1982).

In summary, the present study is the first to measure thermodynamic quantities for the interaction of an opioid agonist with its receptors using an isolated tissue preparation. We report the analysis of the temperature dependence of the dissociation constant using a Van 't Hoff plot for the binding of the highly selective δ ligand DPDPE to the δ-opioid receptor of MVD, using the method of partial irreversible receptor blockade by β-CNA. It appears that the interaction proceeds with a negative change in enthalpy, positive change in entropy and is primarily enthalpy-driven.

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