MOLECULAR PHARMACOLOGY, 14, 540–548

Agonist-Specific Effects of Monovalent and Divalent Cations on Adenylate Cyclase-Coupled *Alpha* Adrenergic Receptors in Rabbit Platelets

BIE SHUNG TSAI AND ROBERT J. LEFKOWITZ[1]

Department of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

(Received January 20, 1978)
(Accepted March 9, 1978)

SUMMARY

TSAI, BIE SHUNG & LEFKOWITZ, ROBERT J. (1978) Agonist-specific effects of monovalent and divalent cations on adenylate cyclase-coupled *alpha* adrenergic receptors in rabbit platelets. *Mol. Pharmacol.*, 14, 540–548.

The *alpha* adrenergic receptors of rabbit platelets can be identified by using [^{3}H]-dihydroergocryptine. The binding of [^{3}H]dihydroergocryptine to platelet lysates reaches equilibrium in 30 min and is slowly reversible. The maximal number of binding sites is 280 ± 25 fmoles/mg, and the dissociation constant was determined to be 2.26 ± 0.48 nM. The specificity of the binding sites for receptor agonists and antagonists is consistent with that of an *alpha* adrenergic response in inhibiting prostaglandin E_1-stimulated adenylate cyclase in the same lysate preparation and is thus in agreement with the classical definition of *alpha* adrenergic receptors in mediating physiological responses. Divalent cations such as magnesium and manganese and monovalent cations such as sodium markedly influence the binding affinity of agonists but have little or no effect on the binding of antagonists. Calcium produces no change in binding. Neither magnesium nor sodium alters the number of receptor sites. Magnesium (1.25 mM) increases the binding affinity of (−)-epinephrine by a factor of 4. In contrast, sodium chloride (100 mM) strikingly decreases the binding affinity of agonists (more than 10-fold). The effect of sodium chloride on agonists is apparent with concentrations as low as 10 mM, and shows a dose-dependent response. LiCl and KCl are only 44% and 19% as effective, respectively, as sodium chloride. The magnitude of the shift in binding affinity of *alpha* adrenergic agents produced by sodium chloride is directly related to the intrinsic activity of those agents for inhibition of prostaglandin E_1-stimulated adenylate cyclase. The results suggest that physiological concentrations of magnesium chloride and sodium chloride may selectively regulate the binding affinity of agonists.

INTRODUCTION

The catecholamines epinephrine and norepinephrine cause platelets to aggregate (1–4). Epinephrine is more potent than norepinephrine as an inducer of platelet aggregation, while isoproterenol is ineffective (1, 5). The *alpha* adrenergic antagonist phentolamine blocks the effect of catecholamines on platelet aggregation, whereas the *beta* adrenergic antagonist propranolol has no effect (1, 3, 5, 6). These data suggest that epinephrine and norepinephrine produce their effects by acting on *alpha* adrenergic

This work was supported by Grants HL 20339 and HL 16037 from the National Institutes of Health and a grant-in-aid from the American Heart Association, with funds contributed in part by the North Carolina Heart Association.

[1] Investigator of the Howard Hughes Medical Institute.

0026-895X/78/0144-0540$02.00/0

receptors.

Activation of platelet *alpha* adrenergic receptors reduces both basal and prostaglandin E_1-stimulated cyclic 3',5'-AMP formation in intact human and rabbit platelets (7–10). There is also a decrease in adenylate cyclase activity in human platelet membrane fractions (8, 11, 12). Although the mechanism by which catecholamines affect platelet aggregation is still unknown, recent studies suggest that it may be associated with the reduction of intracellular cyclic AMP concentration (7, 8, 10).

[^{3}H]Dihydroergocryptine, a potent antagonist of *alpha* adrenergic receptors in human platelets, has been used to label the *alpha* adrenergic receptor in human platelets and platelet lysates (13). The [^{3}H]-DHE[2] binding sites have all the characteristics expected of physiological *alpha* adrenergic receptors. [^{3}H]DHE has also been successfully used to identify *alpha* adrenergic receptors in peripheral and central nervous tissues (14–17).

Although the human platelet *alpha* adrenergic receptor system is potentially useful for clinical studies, animal models are more practical for studies of hormonal regulation of *alpha* adrenergic receptor function. Accordingly, the present studies were undertaken to establish the validity of using [^{3}H]DHE binding to study rabbit platelet *alpha* adrenergic receptors. In the course of these studies, several interesting agonist-specific regulatory effects of mono- and divalent cations on *alpha* adrenergic receptor binding were discovered and form the basis for this report.

METHODS

Preparation of Platelet Lysates

Blood was collected from male New Zealand white rabbits (3–5 kg) by means of ear artery puncture, with 3.2% sodium citrate as an anticoagulant. Some of the rabbits were bled more than once, at 3–4-week intervals, but the results from these rabbits were not different from those bled only once. Platelet lysates were prepared according to the method for human platelets, with some modification (13). Whole blood was centrifuged at 280 × *g* for 10 min at 25°, and the platelet-rich plasma was then centrifuged at 16,000 × *g* for 10 min. No significant contamination by red cells or leukocytes was found in samples prepared in this manner. The platelet pellet was washed twice by resuspension in buffer I solution (Tris-HCl, 0.05 M; NaCl, 0.15 M; EDTA, 0.02 M, pH 7.5) and centrifuged at 16,000 × *g* for 10 min. Suspension of the washed platelets in buffer containing Tris-HCl (0.005 M) and EDTA (0.005 M), pH 7.5, for 1 min at room temperature resulted in lysis. The lysate was homogenized for 20 strokes with a motor-driven, Teflon-tipped pestle and then centrifuged at 39,000 × *g* for 10 min at 4°. For the binding assay, unless otherwise indicated, the resulting pellet was washed once by resuspension in incubation buffer (Tris-HCl, 70 mM, pH 7.5) and centrifuged. The final pellet was ready for the binding assay after resuspension in incubation buffer. When the effects of magnesium chloride, calcium chloride, and manganese chloride on [^{3}H]DHE binding were studied, the lysate pellet was washed once by resuspension in Tris-HCl buffer (70 mM, pH 7.5) containing 1 mM EDTA and centrifuged. The final pellet was resuspended in incubation buffer containing 1 mM EDTA.

Binding Assay

The receptor binding studies were carried out in a final volume of 0.2 ml of incubation buffer (Tris-HCl, 70 mM, pH 7.5) containing 0.8 mM ascorbic acid. For the standard assay, the concentration of [^{3}H]DHE in the incubation medium was 5 nM. The reaction was initiated by the addition of 0.15–0.3 mg of platelet lysate protein and continued for 30 min at 25°. The incubation was terminated by adding 2 ml of incubation buffer and rapidly filtering the mixture under vacuum through Whatman GF/C glass fiber filters. The filters were further washed with 20 ml of incubation buffer at 25°. This wash reduced nonspecific binding without decreasing specific binding. After drying, the filters were placed in a Triton-toluene solution and counted by liquid scintillation spectrometry at an efficiency of 51%. Specific [^{3}H]DHE

[2] The abbreviations used are: DHE, dihydroergocryptine; cAMP, adenosine cyclic 3',5'-monophosphate; PGE_1, prostaglandin E_1.

binding was defined as the difference between counts in the absence and presence of 0.1 mM phentolamine. Binding data described under RESULTS refer to specific binding. When the concentration of [^{3}H]-DHE was 5 nM, the specific binding ranged from 65 to 80% of the total binding.

Adenylate Cyclase Assay

Assays were performed as reported previously (13). The final incubation mixture contained 55 μl of Tris-HCl, 30 mM (pH 7.5); $MgCl_2$, 10 mM; cAMP, 0.1 mM; ATP, 1.5 mM; [α-^{32}P]ATP, 1–2 × 10^6 cpm; phosphoenolpyruvate, 5 mM; pyruvate kinase, 40 μg/ml; and myokinase, 20 μg/ml. Incubations were carried out for 10 min at 37° and terminated by adding 1 ml of a solution containing [^{3}H]cAMP (15,000 cpm), 100 μg of ATP, and 50 μg of cAMP. [^{32}P]cAMP was isolated by the method of Salomon *et al.* (18), and [^{3}H]cAMP was used to monitor the recovery during chromatography. In all assays propranolol (10 μM) was included to block *beta* adrenergic receptors. The basal adenylate cyclase activity was 31.55 ± 3.26 pmoles of cAMP generated per minute per milligram of protein (n = 6). Adenylate cyclase activity was stimulated 10–20-fold by 10 μM PGE_1. The inhibition of the PGE_1-stimulated enzyme by *alpha* adrenergic agonists was then determined. (−)-Epinephrine at 1 mM decreased the PGE_1-stimulated enzyme activity by 20–30%. The "intrinsic activity" of *alpha* adrenergic agents was calculated by dividing the maximal decrement in activity caused by the agent by the decrement produced by 1 mM (−)-epinephrine. Protein was determined by the method of Lowry *et al.* (19).

Materials

[^{3}H]DHE with a specific activity of 23 Ci/mmole was obtained from New England Nuclear. (−)-Epinephrine bitartrate, (−)-norepinephrine bitartrate, (−)-isoproterenol bitartrate, (−)-phenylephrine, dopamine HCl, (±)-propranolol, and dihydroergotamine were purchased from Sigma; (+)-epinephrine bitartrate and (+)-norepinephrine bitartrate, from Sterling-Winthrop; phentolamine HCl, from Ciba; clonidine HCl, from Boehringer/Ingelheim; dihydroergocryptine methanesulfonate, from Sandoz; and methoxamine HCl, from Burroughs Wellcome.

RESULTS

Identification of Alpha Adrenergic Receptors with [^{3}H]DHE Binding

Binding of [^{3}H]DHE to *alpha* adrenergic receptors in several other tissues has previously been described in some detail. Accordingly, although we have extensively characterized [^{3}H]DHE binding to rabbit platelet lysates, these data will be summarized only briefly below. The binding of [^{3}H]DHE (5 nM) to rabbit platelet lysates reached equilibrium in 30 min at 25° ($t_{1/2}$ approximately 10 min) and was slowly reversible ($t_{1/2}$ approximately 120 min). The binding was saturable (Fig. 1), with the maximal number of binding sites determined to be 280 ± 25 fmoles/mg of protein (mean ± standard error of three experiments). Half-maximal binding occurred at 2.26 ± 0.48 nM (three experiments), providing an estimate of the equilibrium dissociation constant. The ability of adrenergic receptor agonists to inhibit [^{3}H]DHE binding is shown in Fig. 2A. The order of potency of catecholamines in competing with [^{3}H]-DHE for the binding sites was found to be (−)-epinephrine > (−)-norepinephrine > (−)-isoproterenol. These findings are consistent with the classical pattern of *alpha* adrenergic receptors in mediating responses induced by catecholamines (20). Serotonin and dopamine were much less effective in competing for the binding sites. Clonidine, a potent *alpha* adrenergic agonist in peripheral vascular smooth muscle, was slightly more potent than (−)-epinephrine (data not shown). In human platelets, clonidine was found to be 17 times more potent than (−)-epinephrine (13). The affinity of the binding sites for catecholamines showed a stereospecific preference, with (−)-epinephrine and (−)-norepinephrine being 9 and 40 times more potent, respectively, than their corresponding (+) isomers. Alpha adrenergic receptor antagonists such as phentolamine, dihydroergocryptine, and DHE strongly compete with [^{3}H]DHE for the binding sites (Fig. 2B). In contrast, the *beta* adrenergic antagonist propranolol was

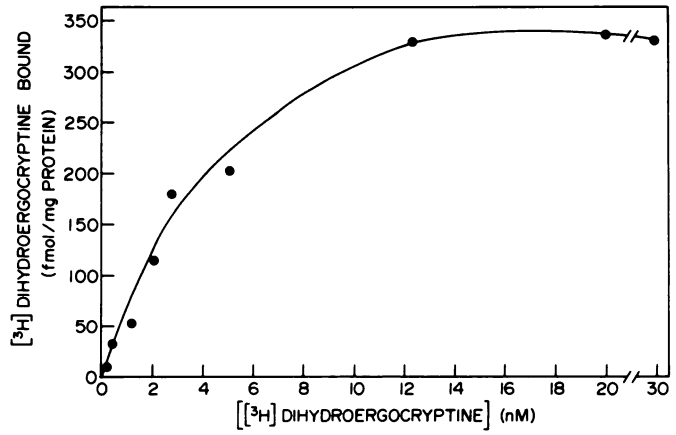

FIG. 1. *Specific binding of [^{3}H]DHE to rabbit platelet lysates as a function of [^{3}H]DHE concentration*
Rabbit platelet lysates were incubated with the indicated concentrations of [^{3}H]DHE, and binding was determined as described under METHODS. Each value is the mean of duplicate determinations. The results are representative of three such experiments.

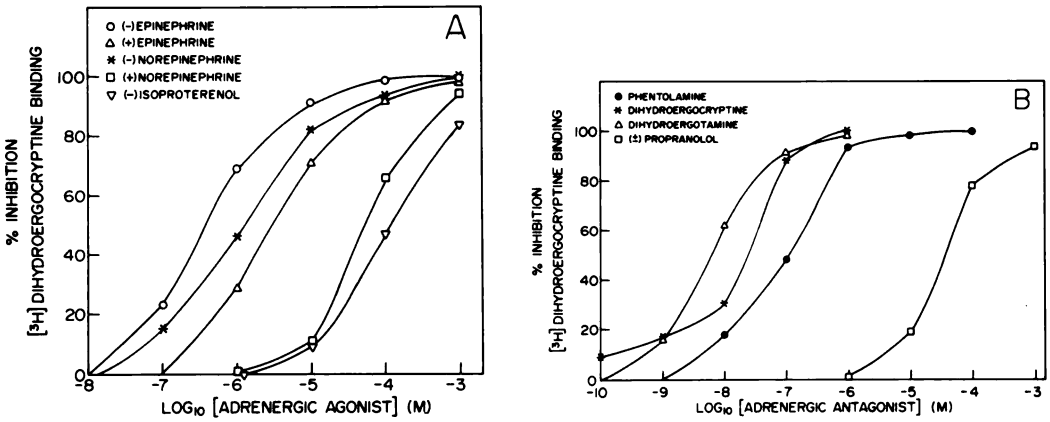

FIG. 2. *Inhibition of [^{3}H]DHE binding by adrenergic agonists (A) and antagonists (B)*
A. Rabbit platelet lysates were incubated in the presence and absence of various concentrations of adrenergic agonists, and specific binding was determined. One hundred percent inhibition refers to the inhibition of specific binding by 0.1 mM phentolamine. Each value is the mean of two to seven duplicate experimental observations. Data from separate experiments deviated from the mean by 10% or less.

B. Rabbit platelet lysates were incubated in the absence and presence of various concentrations of adrenergic antagonists, and specific binding was determined. One hundred percent inhibition refers to the inhibition of specific binding by 0.1 mM phentolamine. Each value is the mean of two to four duplicate experimental observations. Data from separate experiments deviated from the mean by 10% or less.

a very weak inhibitor. The calculated K_D (dissociation constant) values for various drugs tested for the binding sites are shown in Table 1.

Specificity of Adenylate Cyclase Inhibition

(−)-Epinephrine and (−)-norepinephrine are known to inhibit PGE_1-sensitive adenylate cyclase in human platelet membrane preparations (8, 11-13). This response was shown to be effected through *alpha* adrenergic receptors. In the present study, it was found that catecholamines such as (−)-epinephrine also decrease the PGE_1-stimulated adenylate cyclase activity in a dose-dependent manner (Fig. 3). In separate experiments, we have shown that this effect on the enzyme is mediated by *alpha* adrenergic receptors (phentolamine and DHE abolished the response whereas pro-

TABLE 1

Apparent dissociation constants (K_D) of adrenergic agents and other drugs

Dissociation constants were calculated from the following equation (21):

$$K_D = \frac{EC_{50}}{1 + S/K_{DHE}}$$

where K_D is the apparent dissociation constant of the competing agents, K_{DHE} is the equilibrium dissociation constant of [^{3}H]DHE determined from binding studies (2.26 nM), S is the concentration of [^{3}H]DHE present in the binding assay, and EC_{50} is the concentration of competing agents causing 50% inhibition of specific [^{3}H]DHE binding.

Adrenergic drug	Inhibition of [^{3}H]DHE binding (K_D)
	μM
Agonists	
(−)-Epinephrine	0.1
(−)-Norepinephrine	0.4
(+)-Epinephrine	0.9
(+)-Norepinephrine	15
(−)-Isoproterenol	39
Clonidine	0.08
(−)-Phenylephrine	2.5
Methoxamine	28
Dopamine	3.0
Serotonin	3.0
Antagonists	
Dihydroergotamine	0.0019
Dihydroergocryptine	0.006
Phentolamine	0.03
(±)-Propranolol	9.0

pranolol did not). The relative potencies of catecholamines for inhibiting adenylate cyclase activity (Fig. 3) were the same as those for the inhibition of [^{3}H]DHE binding: (−)-epinephrine > (−)-norepinephrine > (−)-isoproterenol. The stereospecificity of adenylate cyclase inhibition by catecholamines was also in agreement with that of [^{3}H]DHE binding sites; (−) isomers of the catecholamines were more potent than their respective (+) isomers. Thus these results further support the contention that the [^{3}H]DHE binding sites are indeed equivalent to the physiological *alpha* adrenergic receptors.

Effects of Cations on Binding Affinities of Agonists and Antagonists

Divalent cations. Calcium ions are required for the platelet aggregation induced by many agents (22, 23) as well as for the expression of various *alpha* adrenergic effects. It has not been known whether calcium is required for binding of catecholamines to platelet *alpha* adrenergic receptors. The addition of 2.5 mM calcium chloride (in excess of 0.5 mM EDTA) to the assay medium resulted in no change in [^{3}H]DHE binding in this study. Competition binding curves for (−)-epinephrine and DHE were obtained in the presence and absence of calcium to determine its effect on binding affinity. It was found that the displacement curves for the agonist and the antagonist were independent of the presence of calcium (data not shown). There was no change in the binding affinity of the agonist or the antagonist with the addition of calcium ions to the medium.

Unlike calcium, magnesium and manganese ions at 1.25 mM (in excess of 0.5 mM EDTA) enhanced the ability of (−)-epinephrine to compete with [^{3}H]DHE for the receptors. Magnesium did not significantly change the binding of [^{3}H]DHE, whereas manganese usually decreased [^{3}H]DHE binding by 10%. In the presence of $MgCl_2$, the displacement curve for (−)-epinephrine was shifted significantly to the left by a

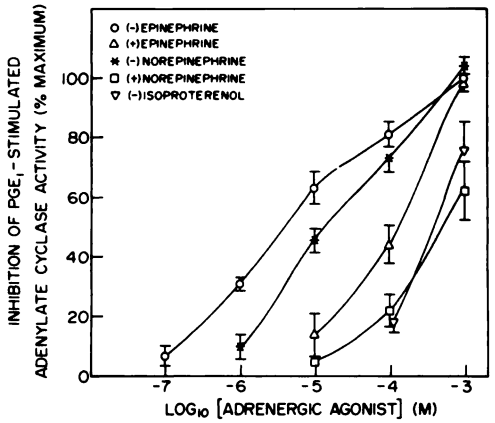

FIG. 3. *Inhibition of PGE_1-stimulated adenylate cyclase activity in rabbit platelet lysates by adrenergic agonists*

Maximal response refers to the decrease in cAMP generation produced by 1 mM (−)-epinephrine. This decrease is a 20–30% reduction in PGE_1-stimulated enzyme activity. Values shown are means and standard errors from three to seven experiments. Assays were performed in triplicate.

factor of 4 (Fig. 4) ($p < 0.01$ by comparing the EC_{50} values in the presence and absence of $MgCl_2$; $n = 4$, paired t-test). By contrast, the displacement curve for DHE did not shift significantly ($p > 0.1$, $n = 3$). The shift to the left in the displacement curve for (−)-epinephrine produced by $MnCl_2$ was variable from one preparation to another (2–10-fold) (data not shown).

Monovalent cations. There was a striking decrease in the ability of (−)-epinephrine to inhibit [^{3}H]DHE binding in the presence of 100 mM NaCl. The displacement curve for (−)-epinephrine was shifted 16-fold to the right (Fig. 5). Neither the binding of [^{3}H]DHE nor the displacement curve for DHE was altered in the presence of NaCl. This finding suggests that sodium ion does not alter the number of receptor sites. The effect of sodium chloride on the binding affinity of (−)-epinephrine was apparent at concentrations as low as 10 mM (producing a 2.4-fold shift) and increased with increasing sodium concentration (Fig. 6). At equal concentrations (100 mM), LiCl was only 44% as effective as NaCl, whereas KCl was only 19% as effective (Fig. 7). When [^{3}H]DHE displacement was studied in the presence and absence of NaCl with other *alpha* adrenergic agents, the results shown in Table 2 were obtained. The binding affinities of (−)-norepinephrine, (−)-isoproterenol, (+)-norepinephrine, and (+)-epinephrine were markedly decreased. In contrast, the displacement curves for clonidine, phenylephrine, and methoxamine were shifted only slightly (about 2-fold) by NaCl. These data indicate that the extent of the sodium-induced decrease in the binding affinities of *alpha* adrenergic agents is not related to the binding affinities of the agents in the absence of added sodium.

In order to determine whether the rela-

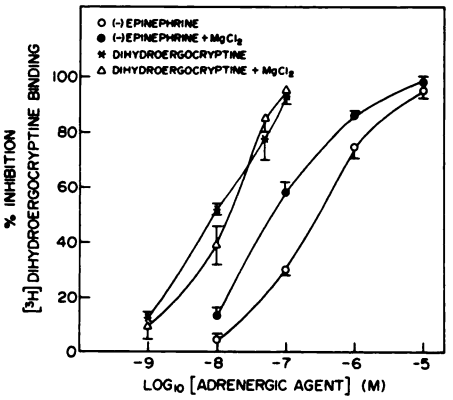

FIG. 4. *Effect of $MgCl_2$ on competition of (−)-epinephrine and DHE for [^{3}H]DHE binding sites in rabbit platelet lysates*

Rabbit platelet lysates were prepared as described in METHODS. Assays were performed in the presence of 1.25 mM $MgCl_2$ in excess of 0.5 mM EDTA. Results shown are means and standard errors of three or four experiments.

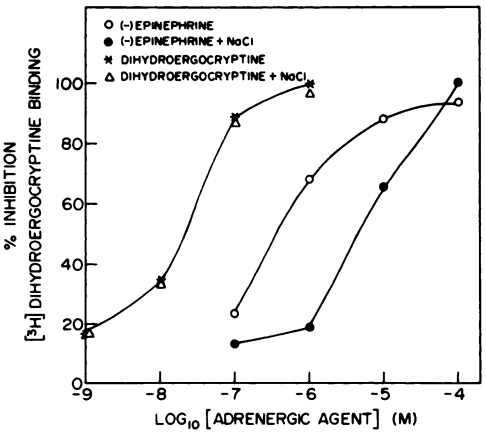

FIG. 5. *Effect of NaCl (100 mM) on competition of (−)-epinephrine and DHE for [^{3}H]DHE binding sites in rabbit platelet lysates*

Results shown are means of three to seven experiments.

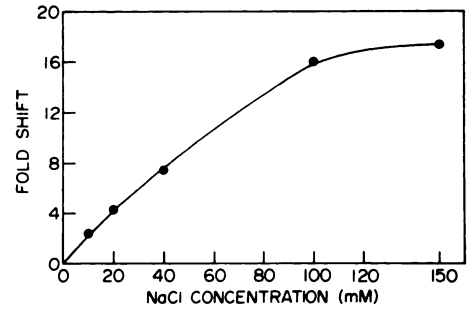

FIG. 6. *Concentration dependence of NaCl-induced shift in (−)-epinephrine displacement curve of [^{3}H]DHE binding*

Results shown are means of two or three experiments. Shifts in the binding displacement curves were determined by comparing the 50% displacement concentrations of (−)-epinephrine in the absence and presence of various concentrations of NaCl. The shift was then determined as the ratio of EC_{50} in the presence of NaCl to the EC_{50} in the absence of NaCl. A "1-fold" shift means that no shift occurred.

TABLE 2

NaCL (100 mM)-induced shifts in [^{3}H]DHE displacement curves, and intrinsic activities of various alpha adrenergic agents as inhibitors of PGE_1-stimulated adenylate cyclase activity

The values shown are means and standard errors of the number of experiments indicated in parentheses. The intrinsic activity of *alpha* adrenergic agonists was determined at a concentration of 1 mM. Dihydroergocryptine, dihydroergotamine, and phentolamine showed no intrinsic activity at concentrations up to 1 μM, 1 μM, and 50 μM, respectively. A "1-fold" shift indicates that no shift occurred.

Agent	Shift in [^{3}H]DHE displacement curve	Intrinsic activity
	-fold	
(−)-Epinephrine	16 ± 1.9 (7)	1.0 (7)
(−)-Norepinephrine	13 ± 5 (2)	1.08 ± 0.7 (5)
(−)-Isoproterenol	11 ± 3.3 (3)	0.43 ± 0.04 (6)
(+)-Epinephrine	12.6 ± 4.0 (2)	1.04 ± 0.04 (5)
(+)-Norepinephrine	10.5 ± 0.5 (2)	0.81 ± 0.04 (2)
Clonidine	2.3 ± 0.7 (2)	0.39 ± 0.07 (4)
(−)-Phenylephrine	2.5 ± 0.5 (2)	0.24 ± 0.04 (4)
Methoxamine	1.9 ± 0.07 (2)	0.16 ± 0.02 (3)
Phentolamine	2.3 ± 0.3 (3)	0 (4)
Dihydroergotamine	1.0 (1)	0 (1)
Dihydroergocryptine	1.0 (3)	0 (4)

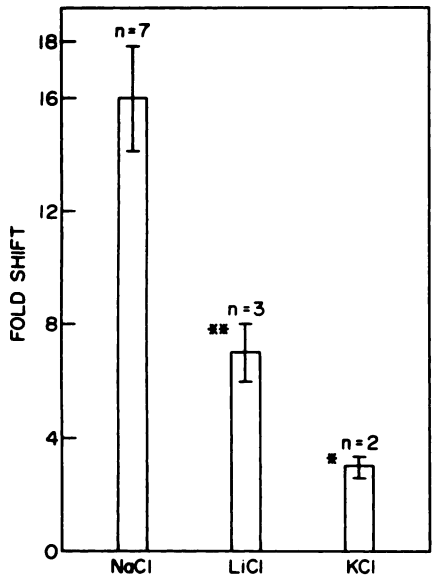

FIG. 7. *Relative effects of NaCl, LiCl, and KCl on shift in (−)-epinephrine receptor binding displacement curve*

Salts were present at a concentration of 100 mM. Results shown are means and standard errors from two to seven experiments.

* $p < 0.05$ compared with LiCl.

** $p < 0.02$ compared with NaCl (unpaired Student's *t*-test).

tive effect of sodium ion on the binding affinities of adrenergic agonists is in some way related to their intrinsic activities as inhibitors of adenylate cyclase, the maximal inhibition of PGE_1-stimulated adenylate cyclase by *alpha* adrenergic agents was studied (Table 2). The shifts in binding affinity of *alpha* adrenergic agents caused by NaCl correlated very well with their intrinsic activities as inhibitors of PGE_1-sensitive enzyme activity.

DISCUSSION

These results show that [^{3}H]DHE binding sites in rabbit platelet lysates have characteristics to be expected of the physiological *alpha* adrenergic receptors that mediate both the inhibition of PGE_1-sensitive adenylate cyclase and platelet aggregation induced by catecholamines. In general, the specificity, stereospecificity, saturability, and kinetics of [^{3}H]DHE binding sites in rabbit platelet lysates are very similar to those found in several other tissues (13–16).

Although calcium has been shown to be required for the platelet aggregation produced by many inducing agents, the present study demonstrates that the interaction between *alpha* adrenergic agonists and the receptors is not dependent on the presence of calcium. It has also been found that calcium has no effect on the binding affinity of rabbit uterine *alpha* adrenergic receptors for agonists and antagonists (24). Magnesium chloride, in concentrations as low as 1.25 mM, enhances the binding affinity of agonists with little effect on the binding of

antagonists, suggesting that endogenous magnesium may play a role in the action of *alpha* adrenergic agonists on platelet aggregation. In a very recent study, physiological concentrations of magnesium chloride have been found to be required for *beta* adrenergic agonists to form a ligand-receptor complex of high affinity in frog erythrocytes (25).

The major finding of the present study is the selective effect of sodium ion on the interaction of *alpha* adrenergic agonists with receptors. Since it is known that the excitatory effect of *alpha* adrenergic receptors in peripheral tissues increases the permeability of plasma membrane to sodium ion (26, 27), the differential effect of sodium on the binding of agonists and antagonists to the *alpha* adrenergic receptor is intriguing and may be somehow related to the ionic mechanisms of *alpha* adrenergic receptor stimulation. So far, little direct evidence is available regarding the exact subcellular localization of *alpha* adrenergic receptors in the platelet. If the receptors are not directly exposed to the circulating fluid, an increase of sodium ion concentration in the immediate vicinity of the receptor sites, resulting from the stimulation of *alpha* adrenergic receptors, may serve as an important negative feedback regulator either to enhance the dissociation of the hormone from receptors or to impede the further association of hormone with receptors.

In contrast with the present findings, sodium has been reported to reduce the binding of *alpha* adrenergic agonists to receptors in rat brain by decreasing the number of receptor binding sites without changing binding affinity (28). Sodium has no effect on the binding of antagonists in the brain. It appears, therefore, that the mechanisms by which sodium reduces *alpha* adrenergic agonist binding are different in the platelet and the brain. The effects of sodium on opiate receptors have been reported to be similar to those found here in platelet *alpha* adrenergic receptors (29).

Although the mechanism underlying the sodium effect is unknown, several possible interpretations may be considered. First, it may be related to the coupling of the receptor to adenylate cyclase. The magnitude of the sodium-induced shift in the binding affinities of agonists is directly related to their abilities to promote the interaction between receptors and adenylate cyclase. It appears that "coupling" of the receptors to the enzyme is necessary in order for sodium to produce its effect. The response is quite remarkable with strong agonists and is induced to a lesser extent with partial agonists. In this respect the effect of sodium on the platelet *alpha* adrenergic receptor resembles that of the guanyl nucleotides on *beta* adrenergic receptors (30). A second notion, consistent with formulations of drug-receptor interactions developed by Greenberg and Snyder (31), is that the sodium effect observed in rabbit platelet lysates is a reflection of the fact that agonists and antagonists bind to distinct and noninterchangeable binding sites, while partial agonists can bind to either site. The agonist binding sites in rabbit platelets might in turn exist in high- and low-affinity states. In the presence of sodium, the high-affinity states. In the presence of sodium, the high-affinity state of the agonist binding sites would be shifted to the low-affinity states. In the presence of sodium, the high-affinity state of the agonist binding sites would be shifted to the low-affinity state, thus decreasing the apparent binding affinity of agonists. Whatever its mechanism, this sodium effect may be useful for discrimination between agonists and antagonists by radioligand binding techniques in drug screening and development programs.

REFERENCES

1. Barthel, N. & Markwardt, F. (1974) *Biochem. Pharmacol.*, **23**, 37–45.
2. Clayton, S. & Cross, M. J. (1963) *J. Physiol. (Lond.)*, **169**, 82P.
3. O'Brien, J. R. (1963) *Nature*, **200**, 763–764.
4. Swank, R. L., Fellman, J. H. & Hissen, W. W. (1963) *Circ. Res.*, **13**, 392–400.
5. Mills, D. C. B. & Roberts, G. C. K. (1967) *J. Physiol. (Lond.)*, **193**, 443–453.
6. Bygdeman, S. & Johnson, O. (1969). *Acta Physiol. Scand.*, **75**, 129–138.
7. Salzman, E. W. & Neri, L. L. (1969) *Nature*, **224**, 609–610.
8. Marquis, N. R., Becker, J. A. & Vigdahl, R. L. (1970) *Biochem. Biophys. Res. Commun.*, **39**, 783–789.
9. Haslam, R. J. & Taylor, A. (1971) *Biochem. J.*,

125, 377–379.
10. Harwood, J. P., Moskowitz, J. & Krishna, G. (1972) *Biochim. Biophys. Acta,* **261,** 444–456.
11. Moskowitz, J., Harwood, J. P., Reid, W. D. & Krishna, G. (1971) *Biochim. Biophys. Acta,* **230,** 279–285.
12. Jakobs, K. H., Saur, W. & Schültz, G. (1976) *J. Cyclic Nucleotide Res.,* **2,** 381–392.
13. Newman, K. D., Williams, L. T., Bishopric, N. H. & Lefkowitz, R. J. (1978) *J. Clin. Invest.,* **61,** 395–402.
14. Williams, L. T., Mullikin, D. & Lefkowitz, R. J. (1976) *J. Biol. Chem.,* **251,** 6915–6923.
15. Tsai, B. S. & Lefkowitz, R. J. (1978) *J. Pharmacol. Exp. Ther.,* **204,** 606–614.
16. Strittmatter, W. J., Davis, J. N. & Lefkowitz, R. J. (1977) *J. Biol. Chem.,* **252,** 5472–5477.
17. Greenberg, D. A. & Snyder, S. H. (1977) *Life Sci.,* **20,** 927–932.
18. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.,* **58,** 541–548.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265–275.
20. Ahlquist, R. P. (1948) *Am. J. Physiol.,* **53,** 586–600.
21. Cheng, Y. C. & Prusoff, W. H. (1973) *Biochem. Pharmacol.,* **22,** 3099–3108.
22. Zucker, M. B. & Borelli, J. (1955) *Fed. Proc.,* **14,** 168.
23. Haslam, R. J. (1964) *Nature,* **202,** 765–768.
24. Williams, L. T. & Lefkowitz, R. J. (1977) *Mol. Pharmacol.,* **13,** 304–313.
25. Williams, L. T. & Lefkowitz, R. J. (1978) *J. Biol. Chem.,* in press.
26. Jenkinson, D. H. & Morton, I. K. M. (1967) *J. Physiol. (Lond.),* **88,** 387–402.
27. Somlyo, A. P. & Somlyo, A. V. (1970) *Pharmacol. Rev.,* **22,** 249–353.
28. Greenberg, D. A., U'Prichard, D. C., Sheehan, P. & Snyder, S. H. (1978) *Brain Res.,* in press.
29. Snyder, S. H. (1977) *N. Eng. J. Med.,* **296,** 266–271.
30. Lefkowitz, R. J., Mullikin, D. & Caron, M. G. (1976) *J. Biol. Chem.,* **251,** 4686–4692.
31. Greenberg, D. A. & Snyder, S. H. (1978) *Mol. Pharmacol.,* **14,** 38–49.