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Complex interactions of agonists with α_1-adrenoceptors in intact cells

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1 The apparent K_i values of (−)-noradrenaline (NA), (+)- and (−)-adrenaline (Ad), phenylephrine and the mono-fluorinated NAs (in position 2, 5 or 6) for α_1-adrenoceptors of intact BC_3H_1 cells labelled with [^{3}H]-prazosin were greatly dependent on the incubation temperature.

2 The EC_{50} values of these compounds for stimulation of the inositol phosphate (IP) accumulation at 37°C were intermediate between their apparent dissociation constants at 2°C ($K_i^{2°}$) and at 37°C ($K_i^{37°}$).

3 The fact that an irreversible blockade of 46% ± 6% ($n = 3$) of the [^{3}H]-prazosin binding sites by phenoxybenzamine reduced the maximal IP-formation induced by NA by 57% ± 5% ($n = 3$) shows that there is a direct coupling between α_1-adrenoceptors and phospholipase C in BC_3H_1 cells.

4 The $K_i^{37°}$s of all agonists tested were in the same range (0.1 to 1 mM) and showed no simple correlation with their EC_{50} values.

5 The $K_i^{2°}$ values for all the agonist correlated linearly with their EC_{50} values but were about 20–100 times lower than the respective EC_{50} values (except for the partial agonist methoxamine). In order to explain this difference, we propose that the apparent high affinity in the cold could be due to an [^{3}H]-prazosin-induced alteration of the active site of the α_1-adrenoceptor, increasing its apparent affinity for catecholamines.

Introduction

Much progress has been made in the last few years in our knowledge of the molecular structure and properties of hormone and neurotransmitter receptors. A great number have been purified and even the primary structure of some of them is known (Noda *et al.*, 1982; Ullrich *et al.*, 1984; Dixon *et al.*, 1986; Kubo *et al.*, 1986; Kobilka *et al.*, 1987; Masu *et al.*, 1987). In order to correlate specific amino acid sequences to the different functional regions of a receptor (for example: hormone-recognition site, or areas interacting with transduction and effector proteins) it is vital to characterize thoroughly the protein in its most natural environment.

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We have already shown that the apparent affinities of noradrenaline (NA) for the [^{3}H]-prazosin binding sites on intact BC_3H_1 cells are much higher in the cold than at 37°C (Sladeczek *et al.*, 1983). Similar results were reported on intact hepatocytes (Lynch *et al.*, 1985). The magnitude of the effect seems to be highly dependent on the radioligand used. K_i values for catecholamine-like agonists determined by competition experiments with [^{125}I]-BE 2254 in intact DDT_1 cells (Hoyer, 1984) were much less temperature-dependent than were the K_i values determined with [^{3}H]-prazosin on BC_3H_1 cells (Sladeczek *et al.*, 1983). On BC_3H_1 membrane preparations the temperature dependency of the K_i for NA, determined by [^{3}H]-prazosin binding was still evident, but was much smaller than that of intact cells (Sladeczek *et al.*, 1983). To our knowledge, such a temperature-dependency of apparent agonist affinities, has not been reported for hepatocyte membranes. However, apparent agonist- and antagonist-affinities determined at 25°C were not the

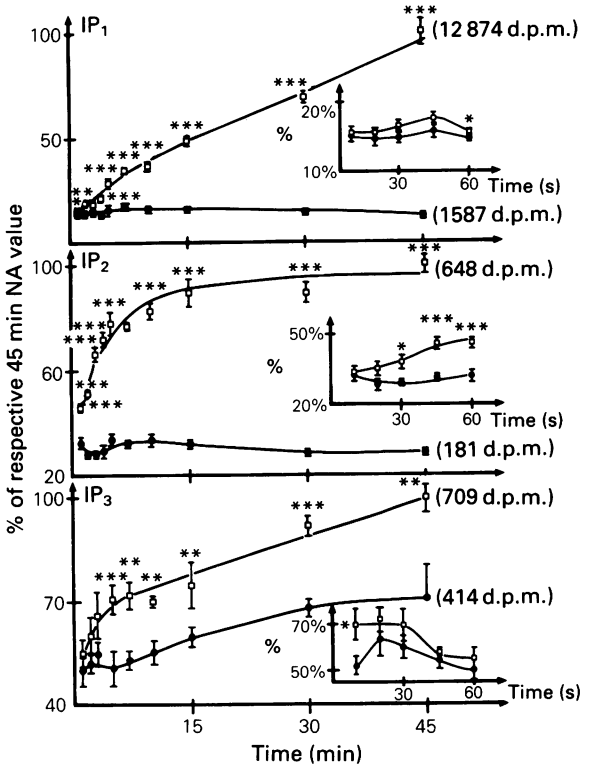

Figure 1 Kinetics of inositol phosphate (IP) formation in intact BC_3H_1 cells: (■) = basal IP formation, (□) = IP formation in the presence of 10 μM noradrenaline (NA). Insets represent the rapid kinetics. Results are expressed as a percentage of the values measured for each class of IP at 45 min in the presence of NA and are the means of at least 6 independent determinations performed on 3 different culture preparations; s.e.mean shown by vertical bars. The significance of the stimulation was determined by Student's t test ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

same in this system, whether [^{3}H]-NA, [^{3}H]-dihydroergocryptine or [^{3}H]-prazosin were used as radioligands (Geynet *et al.*, 1981).

In order to investigate further these non classical receptor drug interactions, we attempted to characterize them on α_1-adrenoceptors in their most natural environment: the intact cell. We compared the agonist K_i values obtained in binding studies with [^{3}H]-prazosin to their EC_{50} values in the IP response. The availability of fluorinated analogues of NA permitted the study and correlation of a series of adrenoceptor agonists (2F-, 5F-, 6F-NA, and NA) having closely related structures combined with markedly different agonist and binding properties (Cantacuzene *et al.*, 1979).

Methods

The BC_3H_1 muscle cell line was a gift from Dr J. Patrick (The Salk Institute, San Diego, CA, U.S.A.). The cells were grown as previously described (Mauger *et al.*, 1982) except that no penicillin or streptomycin sulphate was used. Cells were used 7 to 10 days after seeding without any change of culture medium. Hepatocytes were isolated from the liver of fed female Wistar rats (180–220 g) by collagenase treatment as described by Mauger *et al.* (1984).

[^{3}H]-prazosin binding experiments on intact BC_3H_1 cells were performed as described by Sladeczek *et al.* (1983). Experiments on intact hepatocytes were performed in 3 ml of Eagle's medium supplemented with 2% bovine serum albumin and gassed with 95% O_2:5% CO_2. The reaction was stopped by filtration on Whatman GF/B glass fibre filters followed by three washes with 5 ml of ice cold phosphate-buffered saline. The incubation periods necessary for equilibrium binding were 7 h at 2°C and 45 min at 37°C. Specific binding was defined as the binding displaced by 10 μM of phentolamine.

For IP experiments, cells were seeded in 35 mm plastic dishes in 1.5 ml culture medium containing 3.33 μCi ml^{-1} of [^{3}H]-inositol. Prior to each experiment the culture medium was replaced by HEPES buffered saline and the cells were preincubated for 10 min with 5 mM Li_2SO_4 in order to block IP-degradation (Sherman *et al.*, 1981; Berridge *et al.*, 1982). The HEPES buffer did not contain any inositol. Reactions were started by the addition of ligands, yielding the indicated concentrations in a final volume of 1 ml. Reaction was stopped after 45 min by replacing the incubation medium with 5% perchloric acid (PCA). The IPs were extracted as described by Bone *et al.* (1984) and separated by Dowex anion exchange chromatography (Sladeczek *et al.*, 1988). Extracts of labelled BC_3H_1 cells contained significant quantities of [^{3}H]-inositol and labelled glycerophosphoinositides (GPI), both contaminating the IP-fractions. The following elution protocol allowed us to obtain a good separation of GPI, IP_1, IP_2, and IP_3 fractions: (1) load in 15 ml of 0.5 mM EDTA/5 mM $Na_2B_4O_7$; (2) 3 × 24 ml distilled water; (3) 5 × 4 ml distilled water; (4) 3 × 12 ml 60 mM NH_4HCOO/5 mM $Na_2B_4O_7$; (5) 2 × 24 ml 200 mM NH_4HCOO/5 mM $Na_2B_4O_7$; (6) 1 × 12 ml 200 mM NH_4HCOO/5 mM $Na_2B_4O_7$; (7) 2 × 24 ml 400 mM NH_4HCOO/100 mM HCOOH; (8) 1 × 12 ml 400 mM NH_4HCOO/100 mM HCOOH; (9) 2 × 24 ml 2 M NH_4HCOO/100 mM HCOOH; (10) 1 × 20 ml 2 M NH_4HCOO/100 mM HCOOH.

The radioactivity eluted in steps (5), (7) and (9) corresponded to most of the IP_1, IP_2 and IP_3 respectively. Radioactivity was determined in 4 ml samples. In experiments in which it was not necessary to

Table 1 $K_i^{2°}$, $K_i^{37°}$, EC_{50} and intrinsic activity values for some full and partial adrenoceptor agonists determined on BC_3H_1 cells

	$K_i^{2°}$ (nM)	$K_i^{37°}$ (nM)	EC_{50} (nM)	$EC_{50}/K_i^{2°}$	*Intrinsic activity*
(−)-Ad	2 ± 1	116,200 ± 21,400	240 ± 22	120	1.39
(+)-Ad	100 ± 46	138,200 ± 28,500	4140 ± 1220	41	1.08
(−)-NA	21 ± 3	99,200 ± 17,900	382 ± 64	18	1.00
6F-NA	35 ± 10	93,100 ± 19,700	1240 ± 160	35	1.27
5F-NA	15 ± 8	62,000 ± 25,900	936 ± 205	62	1.04
2F-NA	737 ± 71	82,900 ± 15,500	22,300 ± 700	30	0.37
(−)-Phenylephrine	67 ± 15	12,600 ± 5100	956 ± 213	14	0.59
Methoxamine	36,700 ± 3700	44,280 ± 14,600	73,200 ± 15,000	2	0.40

$K_i^{2°}$ and $K_i^{37°}$ values were calculated by the Cheng-Prusoff (1973) equation with the K_D values of [^{3}H]-prazosin at the respective temperatures (45 pM at 37°C and 26 pM at 2°C) and the drug IC_{50} values determined in competition experiments. Due to the fact that the Hill coefficients of agonist competition curves performed at 37°C were slightly lower than one (0.6–0.9) the $K_i^{37°}$ values are approximate. They were calculated in order to allow a rough comparison between $K_i^{2°}$, $K_i^{37°}$ and EC_{50} values and because we have already shown that it was impossible to fit the agonist competition curves performed at 37°C to a two site model using a classical non-linear curve fit procedure (Sladeczek *et al.*, 1983). EC_{50} values were determined from IP-concentration-response curves. Values are means ± s.e.mean of at least three independent determinations.

separate individual inositol phosphates, only steps (1–4), (9) and (10) were performed and a 4 ml sample from step (9) was counted. This fraction contained most of the IPs and consisted of 90% IP_1, 5% IP_2 and 5% IP_3, in extracts of cells stimulated with 10 μM NA for 45 min.

Results

In our previous study, we showed that the apparent affinity of NA, determined by competition experiments on intact BC_3H_1 cells with [^{3}H]-prazosin, was about 5000 times higher at 2°C than at 37°C. In order to verify that this temperature effect on apparent agonist affinities is a general feature of adrenoceptor agonists, the $K_i^{2°}$ and $K_i^{37°}$ values for various agonists were determined on BC_3H_1 cells with [^{3}H]-prazosin. These values were compared to the EC_{50} values of these drugs for eliciting a cellular response. In order to minimize the influence of amplification steps between receptor occupancy and response, we chose the IP-response for the determination of the EC_{50} values. For the 5-HT_2-receptor-induced IP-formation in WRK_1 cells, we have already shown that there is a linear coupling between receptor occupancy and IP-response (Cory *et al.*, 1987). We have verified that a similar linear coupling occurs for the α_1-adrenoceptor of BC_3H_1 cells. In order to measure the IP-response in the same conditions in which we did the binding experiments at 37°C, we chose a 45 min incubation period. IP_1 accumulation was linear up to this time point and it represented more than 90% of the IPs detected at 45 min (Figure 1). After inactivation of a fraction of the receptors with phenoxybenzamine (Pbz), an alkylating agent known to block irreversibly the α_1-adrenoceptors in this system (Mauger *et al.*, 1982), we observed that a 46% ± 6% ($n = 3$) blockade of the [^{3}H]-prazosin binding sites reduced the maximal IP-formation induced by NA by 57% ± 5% ($n = 3$), without a significant change in the EC_{50}-values (Figure 2). This means that the α_1-adrenoceptor of BC_3H_1 cells is linearly coupled to the IP-response. The EC_{50}-values measured in IP experiments reflect therefore the

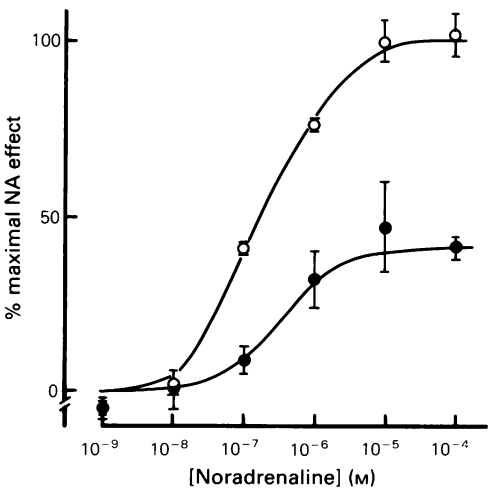

Figure 2 Effect of an irreversible block of a fraction of α_1-adrenoceptor by phenoxybenzamine (Pbz) on noradrenaline (NA) induced IP-formation; (○) control; (●) Pbz 10^{-9} M. α_1-Adrenoceptors of intact BC_3H_1 cells were blocked by Pbz 1 nM as previously described (Mauger *et al.*, 1982). EC_{50} values are 180 nM in the absence and 300 nM in the presence of Pbz.

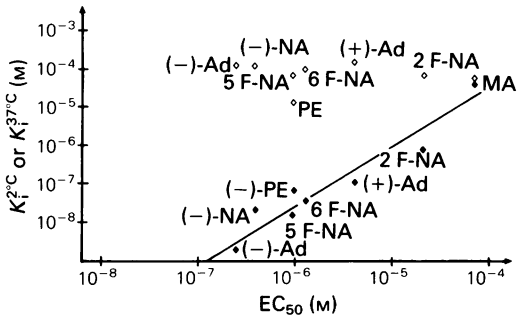

Figure 3 Correlation between (◆) $K_i^{2°}$, (◇) $K_i^{37°}$ and EC_{50} values. Values were taken from Table 1. Abbreviations are: Ad = adrenaline, PE = phenylephrine, MA = methoxamine, NA = noradrenaline. $r = 0.96$, $p = 1.45$.

'real' affinities of this receptor for agonists after an incubation period of 45 min at 37°C.

In Table 1, the EC_{50}, $K_i^{2°}$ and $K_i^{37°}$ values of several full and partial agonists of the α_1-adrenoceptor, are listed. The $K_i^{37°}$ values of these agonists were all within a narrow range (between 10 and 100 μM) in spite of the great differences in their EC_{50}s. On the other hand, $K_i^{2°}$ values correlated well with the EC_{50}s, although all catecholamines displaced [^{3}H]-prazosin at concentrations that were 20–100 times lower in the cold than those required to stimulate the IP-response at 37°C. This correlation is demonstrated graphically in Figure 3. The slope of the correlated line was 1.42, indicating that an agonist with a ten times higher $K_i^{2°}$ value had also an approximately ten times higher EC_{50} for the IP response at 37°C and vice versa.

The use of BC_3H_1 cells for the study of α_1-adrenoceptors is not very widespread. We therefore re-evaluated the temperature effect on apparent agonist affinity observed in this cell line in the more commonly used hepatocyte system. In hepatocytes the equilibrium binding isotherm of [^{3}H]-prazosin was monophasic at high and at low incubation temperatures, indicating the presence of a single class of receptors in either condition. Scatchard analysis of saturation experiments yielded K_D-values of 530 ± 310 pM and 130 ± 50 pM and B_{max} values of 72,000 ± 21,000 sites per cell and 62,000 ± 19,500 sites per cell at 2°C and 37°C respectively (means ± s.e.mean, $n = 4$ in each group). The apparent affinity of the hepatocyte receptor for NA was also very temperature-dependent (Table 2). But there were differences between BC_3H_1 cells and hepatocytes. Hill coefficients differed greatly from unity even at 2°C in hepatocytes (Table 2), whereas in BC_3H_1 cells they were close to unity at this temperature (Sladeczek *et al.*, 1983). In addition, results were much more variable in hepatocytes and the $IC_{50}^{37°}/IC_{50}^{2°}$ ratios were much greater for BC_3H_1 cells than for hepatocytes.

Discussion

This is the first time that rapid formation of IPs has been detected in BC_3H_1 cells. There are indeed two reports describing the formation of IPs in this cell line, but they failed to detect the formation of IPs at short time periods after stimulation (Ambler *et al.*, 1984; 1987). These authors therefore proposed, that the rapid increase of the intracellular Ca^{2+} concentration observed in BC_3H_1 cells (Ambler & Taylor, 1986) might be mediated by something other than IP_3. Our results, however, show that there is no reason to postulate a new Ca^{2+} liberating mediator for BC_3H_1 cells. The kinetics of IP_3 formation shown in Figure 1 are entirely consistent with the well demonstrated second messenger role of IP_3.

There are multiple reasons that could explain why Ambler *et al.* (1984) were not able to observe rapid formation of IPs in BC_3H_1 cells. There are large amounts of [^{3}H]-inositol and [^{3}H]-glycerophosphoinositides in BC_3H_1 cell extracts. The elution protocol for the Dowex anion exchange columns used by Ambler *et al.* (1984) results in very high contaminations of the IP-containing fractions by [^{3}H]-inositol and [^{3}H]-glycerophosphoiniositides. The relatively complicated elution protocol adapted by us allows the contaminating background levels to be reduced sufficiently to detect the small initial

Table 2 Noradrenaline/[^{3}H]-prazosin competition experiments performed on hepatocytes at two different temperatures

Expt. N°	[^{3}H]-*prazosin* (pM)	$IC_{50}^{2°}$ (nM)	$n_H^{2°}$	$IC_{50}^{37°}$ (nM)	$n_H^{37°}$	$IC_{50}^{37°}/IC_{50}^{2°}$
1	85	40	0.47	30,000	0.41	750
2	110	400	0.54	250,000	0.91	625
3	90	200	0.60	20,000	0.36	100
4	95	70	0.58	12,500	0.38	180

Cell viability at the end of each experiment was greater than 90% as determined by their capacity to exclude trypan blue. As Hill coefficients were much lower than 1, even in experiments performed in the cold, we did not calculate the K_i values. Results were obtained from 4 independent experiments.

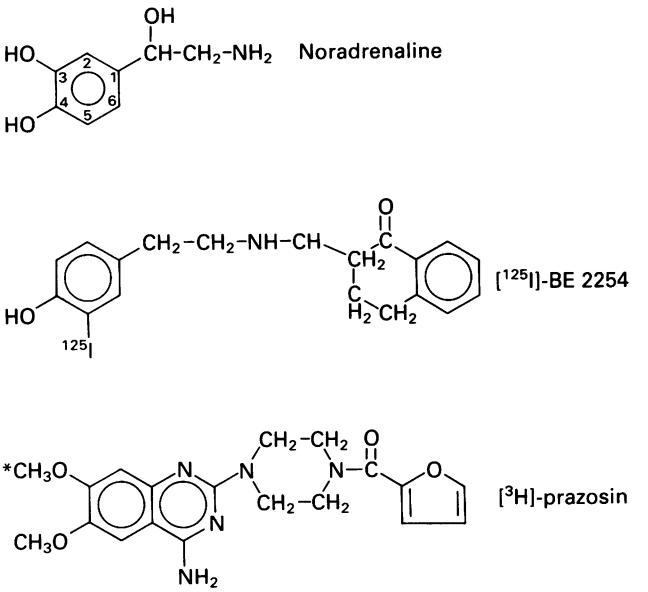

Figure 4 Structure of some of the drugs cited in this study.

increases in IP levels. Another reason for the failure of Ambler *et al.* (1984, 1987) to detect these small changes could be the fact that they used phenylephrine, a partial agonist giving a maximal response only about half as large as NA (Table 1). In addition they labelled confluent BC_3H_1 cells only during 20–30 h, whereas we directly seeded the cells in [^{3}H]-inositol-containing culture medium. Our procedure not only gave much higher agonist-induced IP responses, but it is the only way to be sure that all phosphoinositide pools are in isotopic equilibrium with [^{3}H]-inositol. It is known that different pools of inositol phospholipids exist (Koreh & Monaco, 1986; Monaco, 1987). Slow labelling of the hormone-sensitive pool could render small agonist-induced IP stimulations undetectable when the work is not done under isotopic equilibrium conditions.

Ambler *et al.* (1984) have shown that there is no amplification step between α_1-adrenoceptor occupancy and agonist stimulated incorporation of [^{3}H]-inositol into phospholipids in BC_3H_1 cells. But measuring the labelling of phospholipids can provide misleading estimates of hormone responses because it is well known that Ca^{2+} inhibits the synthesis of phosphatidylinositol from CDP-diacylglycerol and inositol (Berridge & Fain, 1979; Berridge, 1981; Egawa *et al.*, 1981). It was therefore important to determine the relationship between receptor occupancy and the much more direct formation of inositol phosphates in BC_3H_1 cells. The fact that blockade of 47% ± 6% of the [^{3}H]-prazosin binding sites reduced the maximal IP formation induced by NA by 57% ± 5%, without a significant change in the EC_{50} values (Figure 2) clearly shows for the first time that there is no amplification step between phospholipase C and α_1-adrenoceptors in BC_3H_1 cells.

But the most original finding of this paper is, that the EC_{50} values obtained for the IP response at 37°C in intact BC_3H_1 cells were intermediate between the $K_i^{37°}$ and $K_i^{2°}$ values and that a linear correlation exists between the EC_{50} and $K_i^{2°}$ values but not between the EC_{50} and $K_i^{37°}$ values. This suggests that the important difference between $K_i^{37°}$ and $K_i^{2°}$ is due to two distinct phenomena responsible for: (1) the difference between EC_{50} and $K_i^{2°}$ values and (2) the difference between EC_{50} and $K_i^{37°}$ values.

All catecholamines used in the present study were 20 to 100 times more potent in displacing [^{3}H]-prazosin at 2°C than they were in eliciting the IP-response at 37°C (Table 1). But in intact DDT_1 cells the differences between agonist $K_i^{2°}$s (determined in equilibrium competition experiments with [^{125}I]-BE 2254) and EC_{50}s were almost negligible (Hoyer, 1984). A classical interpretation for the high affinity agonist state at 2°C would be that it corresponds to the high affinity agonist site observed in the absence of GTP (Goodhardt *et al.*, 1982; Lynch *et al.*, 1985). This high affinity site would then have α_1-adrenoceptor characteristics only in BC_3H_1 cells, but not in DDT_1 cells. However Lynch *et al.* (1988) recently reported that in hepatocytes the high affinity state of the α_1-adrenoceptor at cold temperature

does not require a G-protein, rendering this interpretation quite improbable. A much more plausible explanation would be that the radioligand used plays a pivotal role in the determination of agonist K_i values. Unfortunately, we were not able to test this hypothesis directly in our system, because non-specific binding of other adrenoceptor ligands such as [^{3}H]-dihydroergocryptine, [^{3}H]-NA or [^{125}I]-BE 2254 was too high. However in DDT_1 cells the only agonist affinity determined by whole cell equilibrium competition experiments at 2°C using [^{3}H]-prazosin and not [^{125}I]-BE 2254 as radioligand was for adrenaline (Toews, 1986). The $K_i^{2°}$ value of 10 nM reported was quite close to the one we found in BC_3H_1 cells (Table 1). It was much lower than the $K_i^{2°}$ of 237 nM found in DDT_1 cells with [^{125}I]-BE 2254 (Hoyer 1984), a value almost identical to the EC_{50} value for the IP response shown in Table 1. It is therefore very likely that the difference between the EC_{50} and $K_i^{2°}$ values of Table 1 are due to the radioligand used.

In order to explain how the radioligand used may or may not induce a high affinity state for drugs acting on catecholamine receptors, we propose the following model. Figure 4 shows the structures of NA, [^{125}I]-BE 2254 and [^{3}H]-prazosin. The ^{125}I containing hydroxyl-phenylethylamine moiety of [^{125}I]-BE 2254 and its side chain up to the amino group closely resembles the structure of NA. The conformational mobility of these parts of both molecules is very similar. [^{3}H]-prazosin on the other hand is a very bulky ligand in which the different groups have very little rotational freedom, forming a fairly rigid, long rod. In view of these stereochemical characteristics, the model in Figure 5 could account for the much lower $K_i^{2°}$ values of catecholamines when competing with [^{3}H]-prazosin rather than ^{125}I-BE 2254. The model represents schematically the interaction of NA or [^{3}H]-prazosin with the catecholamine recognition site of the receptor at 2°C. In order to exhibit specificity it is reasonable to suppose that this region of the receptor has quite stringent structural requirements adapted for catecholamines such as NA (Figure 5a). [^{3}H]-prazosin might then be too bulky to fit well in this structure. Its binding would lead to a slight distortion of the active site region (Figure 5b) and the [^{3}H]-prazosin molecule could bind in such a way that catecholamine-active site interaction could occur even in the presence of the radioligand (Figure 5b,c). The [^{3}H]-prazosin induced stretch would allow a closer interaction of the parts of the catecholamine molecule responsible for binding by formation of hydrogen bonds, ionic or van der Waals interactions with their counterparts on the receptor molecule. This could explain the higher affinity of catecholamines under these conditions. As soon as some

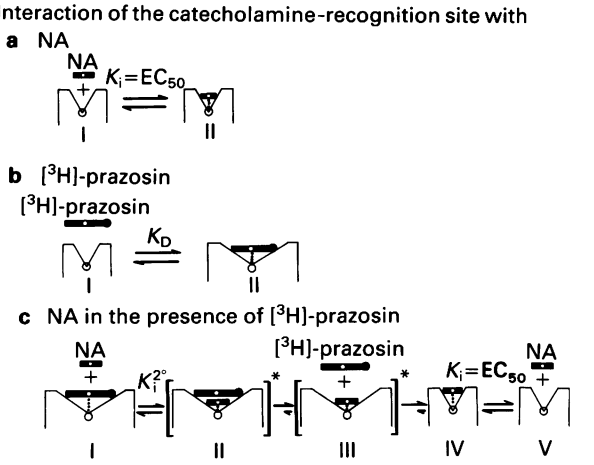

Figure 5 Model proposed for the [^{3}H]-prazosin-induced high affinity state for catecholamine-like agonists. A detailed description is given in the 'Discussion'. The states II and III are transition states and therefore designated with [ ]*.

catecholamine interacts in the presence of [^{3}H]-prazosin with these counterparts of the active site, it neutralizes enough of the [^{3}H]-prazosin binding forces to induce dissociation of the radioligand and the receptor-agonist complex returns to its usual affinity state corresponding to the EC_{50} values. States II and III in Figure 5c represent this reaction schematically. They have to be understood as transition states which might have infinitesimally short life times (Figure 5c). [^{125}I]-BE 2254, due to its great conformational mobility could fit in the catecholamine recognition site of the α_1-adrenoceptor without inducing an active site distortion. This might explain that $K_i^{2°}$ values of agonists determined in DDT_1 cells with this radioligand are almost identical to their EC_{50} values. A detailed study using more or less bulky radioligands and agonists has to be done in order to establish firmly the depicted model.

It is important to note that in the series of agonists shown in Table 1, the catecholamine 2F-NA and the only non-catecholamine tested, methoxamine, exhibit great differences in their $K_i^{2°}$ values despite their very similar EC_{50} values. Both substances are partial agonists with almost identical intrinsic activities. 2F-NA, having nearly the same three dimensional structure as NA, 5F-NA and 6F-NA, has almost the same $EC_{50}/K_i^{2°}$ ratio as these full agonists. Methoxamine is, due to its two methoxygroups in the *trans* position, more bulky than are catecholamines. Its $K_i^{2°}$ value is very close to its EC_{50}. This reinforces the idea that sterical parameters are prob-

ably responsible for the differences between $K_i^{2°}$ and EC_{50} values observed.

At 37°C the high affinity agonist state observed at 2°C seems to be hidden by a second phenomenon responsible for the differences between EC_{50} and $K_i^{37°}$ values. At least three distinct mechanisms could account for the very low apparent afinities of agonists observed at 37°C: (a) an agonist-induced reduction in affinity, (b) an agonist-induced receptor internalisation after which the internalised receptor remains accessible for lipophilic agents such as prazosin but not for the hydrophilic agonists or (c) [^{3}H]-prazosin binds at 37°C not only to the catecholamine recognition site but to an additional site from which it could not be displaced directly by catecholamine like agonists. The elucidation of this phenomenon is the subject of our current investigation. The full understanding at the molecular level of the temperature dependency of agonist-radioligand interactions in the active site of transmembrane receptors could provide important information on the receptor-conformational events occurring during signal transduction.

Our results obtained in intact hepatocytes (Table 2) could give some hint as to the nature of these events. Temperature-dependency of apparent agonist affinities for the α_1-adrenoceptors of intact hepatocytes, assessed by [^{3}H]-prazosin binding, has already been described (Schwarz *et al.*, 1985) but no information on the reproducibility of the effect was given and the described mean effect was only as important as the lowest one found by us (Table 2). It is essential to note that this temperature effect was much lower and more variable in hepatocytes than in BC_3H_1 cells. The same applied to the IP response in these cells. It can therefore be supposed that the importance of the temperature effect depends on the integrity of the receptor-phospholipase C system. Further support for this hypothesis is the fact that the temperature effect described was much smaller in BC_3H_1 cell membranes (Sladeczek *et al.*, 1983) in which we were unable to detect a significant agonist-induced IP-forming activity (Sladeczek & Cory, unpublished results). The active site of a non-functional α_1-adrenoceptor transmembrane signal transduction system (as in membrane preparations) seems therefore to be flexible enough to allow binding of the bulky [^{3}H]-prazosin molecule without inducing the proposed active site distortion.

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