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DIRECT AND INDIRECT EFFECTS OF SULFHYDRYL BLOCKING AGENTS ON AGONIST AND ANTAGONIST BINDING TO CENTRAL α_1- AND α_2-ADRENOCEPTORS

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Abstract—The effects of *p*-chloromercuribenzoate and *N*-ethylmaleimide were evaluated on the binding of (^{3}H)-*p*-aminoclonidine, (^{3}H)-rauwolscine and (^{3}H)-prazosin on rat brain α-adrenergic receptors. Pretreatment of the particulate fraction with increasing concentrations of *p*-chloromercuribenzoate indicated that the binding of all three radioligands was similarly inhibited with an IC_{50} of about 30 μM. This effect was then reduced when agonist [(−)-norepinephrine] or antagonist (phentolamine) were present during the pretreatment. Pretreatment of the particulate fraction at *N*-ethylmaleimide concentrations less than 100 μM specifically decreased the (^{3}H)-*p*-aminoclonidine binding while binding of antagonist was unchanged. *N*-ethylmaleimide produced binding changes similar to those induced by GTP in control membranes, i.e. interconversion of the α_2-adrenoceptors states from a high affinity to a low affinity for agonists. Norepinephrine but not phentolamine reduced the effects of *N*-ethylmaleimide when present during the pretreatment. Taken together, these results suggest that the α_1- and α_2-adrenoceptors possess, within or close by the recognition site, an —SH group which can be blocked at low concentrations by *p*-chloromercuribenzoate but not by *N*-ethylmaleimide. In contrast, the group alkylated by the latter does not seem to be located in the recognition site domain but rather at a site important for the coupling between the α_2-receptor and the GTP-binding protein.

In several receptor systems which mediate the activation of inhibition of adenylate cyclase, it has been reported that agonist and antagonist binding characteristics are differentially affected by divalent cations and guanyl nucleotides [1–4]. These agents are known to modulate the formation of complexes between the receptor and GTP-binding regulatory proteins. In addition to cations and nucleotides, the alkylating agent NEM§ has also been shown to differentiate agonist from antagonist binding to β-adrenergic [3, 5, 7] dopaminergic [8] and muscarinic [9–11] receptors. However, the action of NEM, which presumably alkylates a thiol group, is not identical in each system.

In the case of the β-adrenergic receptor which stimulates adenylate cyclase, simultaneous incubation of the membranes with NEM and an agonist (but not with NEM and an antagonist) leads to a decrease in the number of binding sites.

In fact NEM seems to block a —SH of the GTP binding protein which leads to a blocking of the agonist on the receptor itself, thus suppressing the possibility of interaction with any other ligand [3, 7]. On the other systems which generally mediate inhibition of adenylate cyclase, pretreatment with NEM alone reduces specifically agonist binding. This is the case for the muscarinic and dopaminergic D_2 receptors [8–10]. In these two systems, the reduction in agonist binding by NEM seems to be due to an interconversion of the sites from a high affinity state to a low affinity state for agonists. In fact, NEM action mimics the GTP effect [8, 9], and after NEM treatment guanyl nucleotides cannot affect the apparent affinity for agonists.

In the case of α-adrenergic receptors, the importance and location of essential —SH groups have not been investigated in great detail. On one hand, in uterus [12] and liver [13] membranes it has been demonstrated that the mercurial agent, *p*-hydroxymercuribenzoate, abolishes the binding of (^{3}H)-dihydroergocryptine, a ligand labelling for both α_1- and α_2-adrenoceptors. On the other hand, the binding of (^{3}H)-yohimbine, a specific α_2-antagonist, is not modified significantly by NEM treatment of human platelets [14]. This is surprising since NEM has been reported to block the epinephrine-induced inhibition of adenylate cyclase in this system [14]. We have, therefore, decided to investigate the possible difference between the two —SH blocking agents, PCMB and NEM, on both agonist and antagonist binding to α_1- and α_2-adrenoceptors. In order to avoid differences in tissues and species, this study was performed on rat brain cortex where both receptors are simultaneously present.

MATERIALS AND METHODS

Chemicals. (^{3}H)-PAC; (40.5 Ci/mmole), (^{3}H)-RAU; (87.4 Ci/mmole) and (^{3}H)-PRA; (17.4 Ci/

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§ Abbreviations: NEM, *N*-ethylmaleimide; PCMB, para-chloromercuribenzoate; (^{3}H)-PAC, (^{3}H)-para aminoclonidine; (^{3}H)-RAU, (^{3}H)-rauwolscine; (^{3}H)-PRA, (^{3}H)-prazosin; EDTA, ethylene diamine tetra acetate; DTT, dithiothreitol; NE, (−)-norepinephrine; phen, phene tolamine.

mmole) were purchased from New England Nuclear Corporation (Boston, MA). Parachloromercuribenzoate was obtained from U.S. Biochemical Corporation (Cleveland, OH,), *N*-ethylmaleimide from Fluka A.G. (Buchs, Switzerland), (−)-norepinephrine bitartrate, DL-dithiothreitol and GTP from Sigma Chemical Company (St. Louis, MO). Phentolamine was a gift from Ciba–Geigy (Basel, Switzerland) and (+)-norepinephrine from Hoechst (Frankfurt/Main, F.R.G.). All other chemicals were purchased from commercial sources and of the best available quality.

Membrane preparation. Methods were generally similar to previous central nervous system α-receptor binding studies [15, 16]. Cerebral cortex from male Wistar rats of 180–250 g were homogenized in 30 vol (v/w) of ice-cold Tris–buffer (50 mM Tris–HCl pH 7.0 at 25°) containing 5 mM EDTA with a Brinkmann polytron setting 6 for 30 sec. The homogenate was centrifuged twice at 50,000 *g* for 10 min with intermediate rehomogenization in the same buffer. Since fresh or frozen cerebral cortex gave similar results, the membranes prepared throughout this study were obtained from frozen tissue. Platelet membranes were prepared according to Insel *et al.* [17].

PCMB and NEM treatments. Pellets were resuspended in 60 vol (v/w) of the original wet weight tissue of Tris–EDTA buffer (50 mM Tris–HCl with 0.5 mM EDTA pH 7.0 at 25°) to give about 0.8 mg of protein/ml. Preincubation with or without various concentrations of the sulfhydryl blocking agents was carried out for 10 to 20 min as indicated. The PCMB treatment was stopped by diluting 3 to 8 times the preincubation volume with ice-cold Tris–EDTA buffer followed by a centrifugation at 50,000 *g* for 10 min. Preincubation with NEM was stopped by adding Tris–EDTA buffer containing DTT to give a final concentration of 2 mM DTT. Five minutes later, the suspension was centrifuged at 50,000 *g* for 10 min.

Protection experiments. Protection experiments were performed at the same tissue concentration as described for PCMB and NEM treatments. However, membrane preparations were incubated 20 min at 25° with the protective agents, Phen or NE, before the addition of PCMB and NEM to a final concentration of 100 μM. Incubation with sulfhydryl reagents was carried out for 10 min and terminated as described above followed by extensive washing. This procedure consisted of three additional centrifugations with intermediate rehomogenisation in 200–300 vols (v/w) and a 10 min incubation at 25° between each centrifugation. This extensive washing produced a 30–35% reduction of the total (^{3}H)-PAC binding due to the loss of binding sites and protein content.

Binding assays. Membrane pellets obtained after the pretreatment described above were resuspended in 100 vol (v/w) of Tris–EDTA buffer of the original wet weight tissue. (Protein concentration varied from 0.5 to 0.3 mg/ml depending on the tissue pretreatment.) Incubation tubes, while kept in ice, received 50 μl of diluted radioligand, and different volumes of the membrane suspension [1.66, 1 and 0.5 ml for (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA, respectively]. When necessary, NE dissolved in 0.1% of ascorbic acid and GTP 1 mM in Tris–EDTA were added in a 20 and 100 μl volume respectively. The final incubation volume was adjusted to 2 ml with Tris–EDTA buffer except in (^{3}H)-PAC saturation experiments where the volume was reduced to 1 ml while maintaining the same concentrations of added agents. Samples were incubated for 30 min [(^{3}H)-PAC) or 45 min (^{3}H)-RAU and (^{3}H)-PRA] at 25° and the assays were terminated by rapid filtration under reduced pressure over Whatman GF/B filters. The filters were rinsed three times with 5 ml Tris–EDTA buffer at 25°, transferred to vials containing 6 ml of Aquassure (New England Nuclear, Boston MA) and subsequently counted by liquid scintilation spectrometry at an efficiency of 45–50%. Non-specific binding was determined in parallel samples containing 100 μM (−)-NE for (^{3}H)-PAC and (^{3}H)-RAU and 10 μM Phen for (^{3}H)-PRA binding. The direct effects of PCMB and NEM and competition experiments were conducted at a concentration of about 0.4, 0.5 and 0.1 nM for (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA corresponding to a specific binding of about 900, 1300 and 700 cpm respectively. This specific binding represented in the control tissue 75–90% of the total binding for each radioligands. For the protection experiments the concentrations of (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA were increased to about 0.6, 0.6 and 0.15 nM giving a specific binding of 600, 800, and 450 cpm respectively. In these experiments the specific binding of the control value represented 65 and 80% of the total binding. Protein concentrations were determined by the method of Lowry *et al.* [18] using serum albumin as standard. All results are expressed as the mean ± the standard error of the number of experiments performed.

RESULTS

Effects of PCMB on α-adrenergic radioligand bindings. A 20 min preincubation of the rat brain particulate fractions with increasing concentrations of PCMB leads to a quantitatively comparable inhibition of the binding of three radioligands; (^{3}H)-PAC and (^{3}H)RAU on α_2-adrenoceptors and (^{3}H)-PRA on α_1-adrenoceptors (Fig. 1). A reduction of 50% of the control binding was obtained at a concentration of about 30 μM PCMB. The shape of the inhibition curves was very steep and the total effect of PCMB occurred within a concentration range of less than one order of magnitude. The great similarity of the effect of PCMB, including the rate of inactivation (data not shown), on α_1- and α_2-adrenoceptors in favour of a common mechanism for inhibition of both receptors. In order to test the possibility that the recognition site itself is blocked, protection experiments were performed by using the non-selective agonist (−)-norepinephrine (NE) at a concentration of 10 μM and the non-selective antagonist phentolamine (Phen) at 1 μM. These relatively high concentrations of the reversible protective agents (around 100 times their K_D values) were chosen in order to very rapidly saturate the recognition site. Indeed it is necessary to "shorten" the period during which the receptor is unoccupied and thus accessible to the irreversible inactivation by PCMB. In these experiments, the protective agents

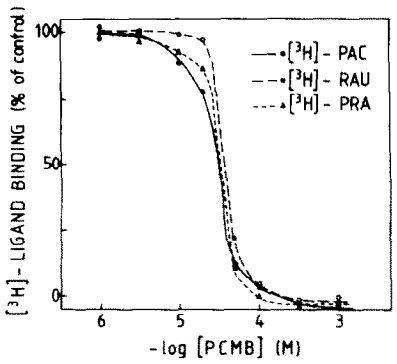

Fig. 1. Effect of PCMB pretreatment on the direct binding of (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA to α_2- and α_1-adrenoceptors. Particulate fractions were pretreated with increasing concentration of PCMB for 20 min at 25°. The membranes were diluted with ice-cold buffer and centrifuged at 50,000 *g* for 10 min. Binding assays were performed on the resuspended pellets as described under materials and methods. Data shown are expressed as the percentage of the control binding and are from one experiment which is representative of the three performed. Control binding values refer to (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA specific bindings in absence of drug and were 22, 17 and 35 fmoles per assay respectively.

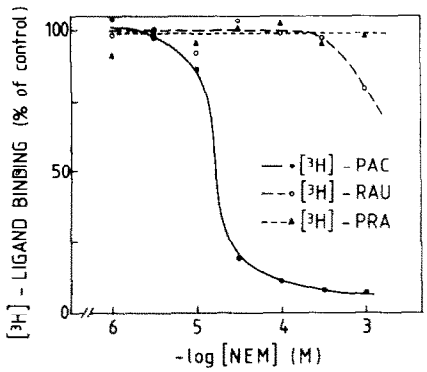

Fig. 3. Effect of NEM pretreatment on the direct binding of (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA. Particulate fractions were pretreated with increasing concentrations of NEM for 20 min at 25°. The membranes were diluted with ice-cold buffer containing DTT (to obtain a final concentration of 2 mM) and centrifuged at 50,000 *g* for 10 min. Binding assays were performed on the resuspended pellets as described under Materials and Methods. Data shown are from one experiment which is representative of the three performed. Data are expressed as the percentage of the control bindings which were in absence of drug 24, 17 and 39 fmoles per assay respectively.

were added to the membranes 20 min before the addition of PCMB, and incubation was carried out for 10 min (Fig. 2). The data indicated that both NE and Phen protected α_1- and α_2-ligand binding from PCMB inactivation. However, the protection was more pronounced on the antagonist binding [(^{3}H)-RAU and (^{3}H)-PRA] than on agonist binding [(^{3}H)-PAC]. The most striking protection was obtained by phentolamine on the (^{3}H)-PRA binding where the effect of PCMB was virtually abolished.

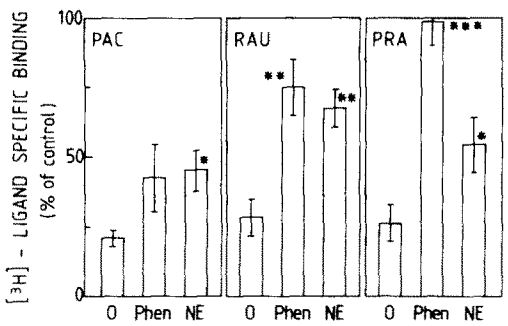

Fig. 2. Protection by (−)-norepinephrine and phentolamine against the action of PCMB treatment. Particulate fractions were incubated for 20 min at 25° without any agents (–○–) or with 10^{-5} M norepinephrine (NE) or 10^{-6} M phentolamine (Phen). PCMB (10^{-4} M) was added and the incubation was continued for 10 min. After washing three times (see Materials and Methods) membranes were assayed for the binding of (^{3}H)-PAC, (^{3}H)-RAU and (^{3}H)-PRA. Data are the means ± SEM from 4 separate experiments and are expressed as the percentage of the binding seen in control membranes (no protective agents, no PCMB) since protective agents alone (no PCMB) had no significant effects by themselves. Significantly different from PCMB alone treated value: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$.

Effect of NEM on the α-adrenergic radioligand bindings. A 20 min preincubation of the rat brain particulate fractions with increasing concentrations of NEM, revealed that this alkylating reagent preferentially affected the binding of the α_2-agonist (^{3}H)-PAC (Fig. 3). Thus, at 10^{-4} M NEM the binding of either (^{3}H)-RAU or (^{3}H)-PRA was unchanged whereas about 90% of the (^{3}H)-PAC binding was abolished. This effect of NEM on the α_2-agonist binding occurred at low concentrations; half of the maximal inhibition was reached at a concentration of about 20–30 μM. Here also, the major effect of NEM was obtained over a narrow range of concentrations. The NEM-agonist specific effect is not limited to brain α_2-adrenergic receptors since a similar pattern was observed on NEM-pretreated human platelet membranes (data now shown). The kinetic of (^{3}H)-PAC binding inactivation by 100 μM NEM on brain α_2-adrenoceptor exhibited two components (Fig. 4). The first, which affected 80–90% of the (^{3}H)-PAC binding in experimental conditions was rapid ($t_{\frac{1}{2}}$ = 1 min) and was mainly responsible for the decrease seen in Fig. 3. After completion of this first inactivation process, the remaining binding component was quite stable.

Effect of NEM on high and low affinity states of α_2-adrenergic receptors: Comparison with the effect of GTP. Since (^{3}H)-PAC labels mainly the high affinity state for agonist of the α_2-adrenoceptor, it is possible that the decrease in (^{3}H)-PAC binding reflects only an interconversion of the high affinity state to the low affinity state similar to that seen with GTP [2, 15]. In order to explore this possibility, binding experiments at steady state, with increasing concentration of (^{3}H)-PAC were performed on membranes pretreated with or without NEM. The results depicted in Fig. 5 indicate that NEM pretreatment reduced the number of the high affinity binding sites in a dose dependent manner. Thus in Fig. 5,

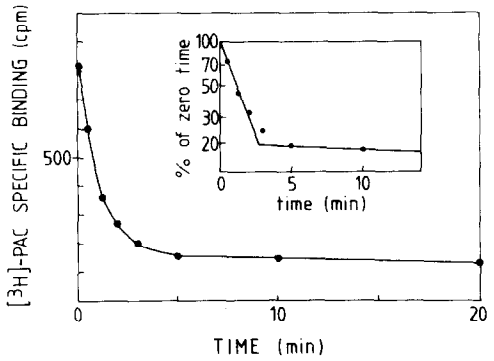

Fig. 4. Kinetics of inactivation of (^{3}H)-PAC binding by 100 μM NEM. Particulate fractions were pretreated with 10^{-4} NEM for the indicated period of time at 25°. Pretreatment was terminated by dilution of the suspension in a Tris–buffer solution, containing DTT at a final concentration of 2 mM. Binding assays were carried out on washed membranes as described in Materials and Methods with 0.45 nM (^{3}H)-PAC. Inset: Semilogarithmic representation of the same binding data. Data shown are from one experiment and representative of the three performed.

Scatchard analysis revealed that the high affinity binding sites decreased from 185 fmoles/mg of protein in control membranes to 140 and 85 after pretreatment with 10 and 20 μM NEM respectively. At the same time there was also a slight decrease in the affinity of (^{3}H)-PAC from 2.4 to 3.5 and 3.6 nM for NEM pretreated membranes. Although the experiment shown in Fig. 5 was qualitatively representative of two others, it should be emphasized that the loss of the high affinity sites at a crucial NEM concentration (i.e. from 10 to 30 μM) varied considerably from one experiment to another. However with 100 μM NEM the saturation isotherm was similar to that observed on control membranes when 100 μM GTP was present in the binding assay.

In order to determine if the loss of the high affinity sites could be attributed to their interconversion to low affinity sites, competition experiments between (−)-NE and (^{3}H)-RAU were performed using membranes pretreated with and without 100 μM of NEM in the presence and in the absence of GTP. In control conditions (no NEM, no GTP), the curves obtained were shallow as indicated by a pseudo-Hill coefficient of 0.55 (Fig. 6). This indicates that under control conditions, (^{3}H)-RAU labelled a population of binding sites having different affinities for the agonists.

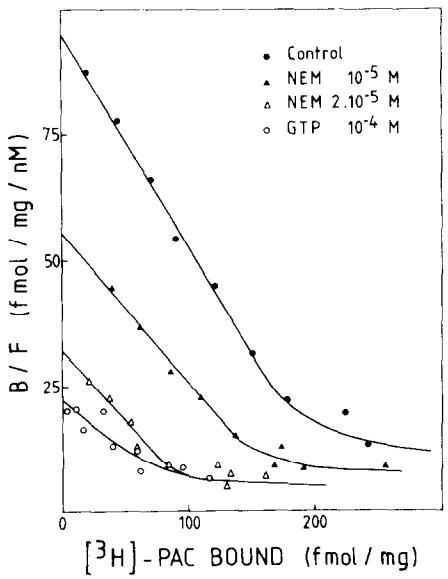

Fig. 5. Effect of NEM pretreatment and GTP on direct agonist (^{3}H)-PAC binding. Particulate fractions were pretreated with none (control) (●), 10^{-5} M NEM (▲), and 2×10^{-5} M NEM (△) for 20 min at 25°. After the washing procedure, described under Materials and Methods, binding assays were carried out with concentrations of (^{3}H)-PAC ranging from 0.2 to 28 nM. For comparison, saturation binding isotherm in the presence of 10^{-4} M GTP (○) was also performed on control membranes. The results were plotted according to the method of Scatchard. Data shown from one experiment which is representative of the three performed.

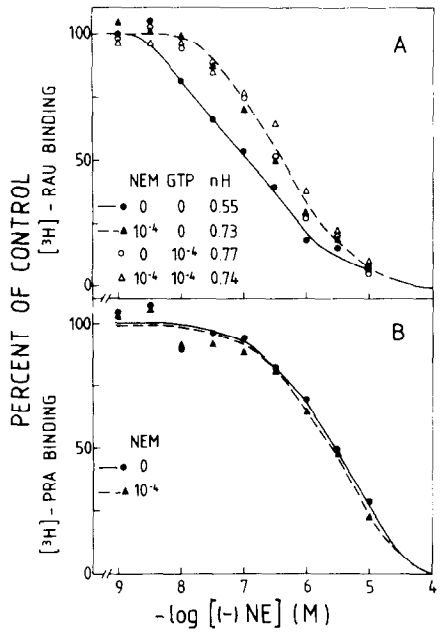

Fig. 6. Comparison of the effects of NEM pretreatment and the presence of GTP on the ability of (−)-norepinephrine to compete for (^{3}H)-RAU and (^{3}H)-PRA. Particulate fractions pretreated with (▲, △) or without (●, ○) NEM for 20 min at 25° were washed and resuspended as described under Materials and Methods. (A) Membranes were incubated with (^{3}H)-RAU and increasing concentrations of (−)-NE in the presence of 10^{-4} M GTP (open symbols) and in the absence of GTP (closed symbols). All curves are from a single experiment that was representative of three to five experiments. In these experiments, pseudo-Hill coefficient mean values ± SEM (N) were in the different conditions: (●) 0.59 ± 0.02 (5); (▲) 0.68 ± 0.05 (5); (○) 0.75 ± 0.01 (3); (△) 0.73 ± 0.03 (3). (B) Membranes pretreated with (▲) or without (●) NEM were incubated with 0.1 nM (^{3}H)-PRA and increasing concentration of (−)-NE. Data shown are from one experiment which is representative of the two performed. Control binding values refers to (^{3}H)-RAU and (^{3}H)-PRA bindings in the absence of drug, and were 12 and 42 fmoles per assay respectively.

In the presence of GTP the competition curve was shifted to the right and was steeper; the pseudo-Hill coefficient was 0.77. Therefore GTP lowered the overall affinity for agonist but did not convert all the sites to an homogeneous state of affinity. Similar results were obtained when (^{3}H)-yohimbine was used to label brain α_2-adrenergic receptors [15]. NEM pretreatment of membranes produced the same effect as GTP (Fig. 6.). Furthermore the GTP and NEM effects were not additive.

Protection by α-adrenergic ligands of (^{3}H)-PAC binding from NEM inactivation. In contrast with what was observed in PCMB experiments, the α-adrenergic antagonist phentolamine had no protective effect. In the same set of experiments (−)-NE (10 μM) could significantly decrease the NEM-induced binding inhibition (Table 1). However, in another set of protection experiments erratic results were obtained with NE which sometimes did not protect against the loss of (^{3}H)-PAC binding. Such variability in the agonist protection was already reported with NG 108–15 membranes for the effect of NEM on opiate receptor [19]. At the present time no explanation for these observations on α_2-adrenoceptor and opiate receptor could be given but they are restricted to agonists while antagonists never protect from NEM action. When present, the partial protection by the agonist NE appears to be related to its affinity for the receptor since at 1 μM concentration the protection afforded by the (+)-NE stereoisomer was smaller than with the (−)-NE isomer (Table 1).

DISCUSSION

In agreement with previous work on the uterus [12] and on the liver [13] this study shows that mercurial agents also inhibit the binding of radioligands to rat brain α-adrenergic receptors. Considering the α_1-adrenoceptors, PCMB seems to block a —SH group located in or close to the recognition site itself, indicated by the fact that agonist and antagonist gave a substantial protection. This appears also to be the case for the α_2-adrenoceptors since protection by norepinephrine and phentolamine is substantial at the (^{3}H)-RAU binding sites. However, the protection is less efficient for the agonist (^{3}H)-PAC suggesting that PCMB might affect another —SH group (s) not directly located in the site itself. This additional action of PCMB could modulate indirectly the agonist binding similar to that seen with NEM (see below).

Table 1. Protection by phentolamine (−)- and (+)-norepinephrine against the action of NEM on (^{3}H)-PAC binding

Drugs	N	(^{3}H)-PAC binding (%) No NEM	NEM pretreated
none	7	100	24.2 ± 1.4
Phen[a] 10^{-6}	4	100.7 ± 3.9	22.2 ± 2.8
(−)-NE[a] 10^{-5}	5	100.9 ± 1.0	40.6 ± 1.6**
(−)-NE[b] 10^{-6}	3	103.3 ± 2.8	58.9 ± 4.2**
(+)-NE[b] 10^{-6}	3	101.7 ± 3.5	35.1 ± 3.6

Particulate fractions were incubated for 20 min at 25°, without any agents or with phentolamine, (−)- or (+)-norepinephrine at the indicated concentrations. NEM was added to a final concentration of 10^{-4} M and the incubation was continued for 10 min. After extensive washing (^{3}H)-PAC binding assay was conducted as described in Materials and Methods. Data are the mean ± SEM of the number of experiments indicated and are expressed as a percentage of the binding in control membranes.

[a] Data from the same set of experiments (see text).

[b] Data from another set of experiments in which (^{3}H)-PAC binding after NEM treatment alone (no Phen nor NE) was 27.4 ± 3.2% of the control value.

** Significantly different from NEM alone treatment membranes values $P < 0.01$.

The action of NEM is very different from that of PCMB since at low concentrations it affects selectively the binding of partial [(^{3}H)-PAC] as well as full α_2-adrenergic agonists. If one excludes *a priori* that agonist and antagonist recognition sites are completely distinct entities, two simple hypotheses could be presented to explain these results.

(1) Both PCMB and NEM block a thiol directly localized on the recognition site, but, due to a difference in the steric hinderance, the presence of PCMB on this site blocks agonist and antagonist binding, whereas the presence of NEM blocks only agonist binding. This is not the case, since pretreatment with NEM does not prevent the further action of PCMB on antagonist binding (data not shown).

(2) NEM in contrast to PCMB does not affect the thiol group located on the recognition site but another nucleophilic group. Its blockade would indirectly modulate agonist binding, leaving antagonist binding unaffected. A good argument in favour of this hypothesis would be that phentolamine did not protect the (^{3}H)-PAC binding at all from NEM inactivation (Table 1). Before postulating the location of the —SH blocked by NEM, it is interesting to recall the characteristics of agonist binding inactivation by NEM. Both saturation and competition experiments suggest that the decrease of the (^{3}H)-PAC binding corresponds to a reduction in the high affinity state of the receptor for agonist (Figs. 5 and 6). The fact that the (^{3}H)-RAU binding is unchanged suggests that the reduction of these high affinity interactions is not due to a simple disappearance of the binding site but rather to an interconversion of the high affinity state to the low affinity state, this low affinity state being unidentified by (^{3}H)-PAC under particular assay conditions used. Furthermore, NEM and GTP have equivalent and non-additive effects on agonist binding suggesting a similar mechanism of action at some point. Indeed, it has been suggested that α_2-adrenoceptors, as other receptors coupled with adenylate cyclase, can interact with a GTP binding protein to form a complex of high affinity for agonist [1, 20, 21]. The presence of GTP dissociates this complex leading to a reduction of the receptor affinity for agonist [20]. Therefore, it is reasonable to propose that as GTP, the alkylation of a thiol group impairs the receptor–GTP binding protein complex. This hypothesis is further strengthened by the fact that agonists which are known to stabilize the complex between the

receptor and the GTP binding protein [22], partially protect (^{3}H)-PAC binding from NEM inactivation, whereas antagonist do not. The "locking" of the α_2-adrenoceptor into its low affinity state for agonist induced by NEM-pretreatment reported here, is similar to what has been observed for D_2 dopaminergic [8] muscarinic [9, 10], Ri adenosine [23] and delta opiate [19] receptors. It is interesting to note that all these systems mediate inhibition of the adenylate cyclase [14, 24–28]. For some of them, low concentrations of NEM also suppress the adenylate cyclase inhibition induced by their respective agonists on α_2-adrenoceptors in human platelet [14] and adipocytes [27] muscarinic heart receptors [28] and at D_2 dopaminergic receptor in anterior pituitary (Bockaert, in preparation). This suggests that the formation of the high affinity state of the receptor, is an essential step in the mechanism of adenylate cyclase inhibition, as it seems to be an essential step in adenylate cyclase stimulation [20].

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Note added in proof: Since this manuscript was submitted, Limbird and Speck (*J. Cyclic Nucleotide Res.* **9**, 191, 1983) have shown similar effects of NEM in the agonist binding to human platelet α_2-adrenoceptor.

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