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THE EFFECT OF THE PLASTICIZERS TBEP (TRIS-(2-BUTOXYETHYL)-PHOSPHATE) AND DEHP (DI-(2-ETHYLHEXYL)-PHTHALATE) ON β-ADRENERGIC LIGAND BINDING TO α_1-ACID GLYCOPROTEIN AND MONONUCLEAR LEUKOCYTES*

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Abstract—The plasticizers tris-(2-butoxyethyl)-phosphate (TBEP) and di-(2-ethylhexyl)-phthalate (DEHP) and the β-adrenergic receptor-blockers [^{3}H]-(−)-dihydroalprenolol ([^{3}H]-(−)-DHA) and [^{3}H]-(−)-CGP 12177 were tested for their ability to interact with β-adrenergic binding to α_1-acid glycoprotein (AAG) and mononuclear leukocytes (MNL). The IC_{50}-values, obtained by displacement of [^{3}H]-(−)-DHA bound to AAG, were 3.5 nM, 2 μM and 4 μM for TBEP, (−)-alprenolol and DEHP, respectively. (±)-CGP 12177 had virtually no effect on radioligand binding to AAG. The [^{3}H]-(−)-CGP 12177 binding to MNL consisted of β-adrenergic receptor binding (K_d = 210 pM) and non-saturable binding. [^{3}H]-(−)-DHA was bound to two different classes of binding sites on MNL, the β-adrenergic receptors (K_d = 440 pM) and a secondary class of binding sites (K_d = 64 nM). (±)-CGP 12177 displaced about 30% of [^{3}H]-(−)-DHA from MNL with an IC_{50}-value of 190 pm. (−)-ALP displaced about 85% of total bound radioligand and gave a biphasic displacement curve with IC_{50}-values of 320 pM and 690 mM, respectively. TBEP displaced a considerable fraction of [^{3}H]-(−)-CGP 12177 and [^{3}H]-(−)-DHA bound to MNL β-adrenergic receptors, whereas DEHP had no effect. In contrast, DEHP caused displacement of [^{3}H]-(−)-DHA from the MNL low affinity sites, but was a markedly less potent displacer compared to TBEP. The present study shows that TBEP and DEHP interact with β-adrenergic transport proteins, non-specific tissue binding sites and β-adrenergic receptors coupled to adenylate cyclase. Plasticizers may thus affect the biology and pharmacology of the β-adrenergic signal system.

The binding sites of AAG and the β-adrenergic receptor have some properties in common. Both MNL β-adrenergic receptors and AAG possess a binding site characterized by stereospecificity, and with higher binding affinity for antagonists than for the agonists [1–6].

The cellular binding of β-adrenergic ligands comprises specific (receptor) binding and non-specific binding. The fraction of the hydrophilic radioligand [^{3}H]-(−)-CGP 12177 non-specifically bound to intact MNL is small [7]. In contrast, the non-specific binding of the lipophilic radioligand [^{3}H]-(−)-DHA consists of saturable low affinity binding in addition to the non-saturable binding [1, 8, 9]. However, the nature of the low affinity class of MNL [^{3}H]-(−)-DHA binding sites is unknown, but it has been suggested that these binding sites may represent AAG adsorbed to the cells [9].

The plasticizers TBEP and DEHP cause a pronounced and selective displacement of basic drugs bound to AAG [10–13]. The present work was conducted primarily to determine whether TBEP and DEHP affect the β-adrenergic ligand binding to MNL in the same way as that to AAG. Secondly, this study was undertaken to characterize the effect of TBEP and DEHP on the different classes of MNL β-adrenergic binding sites.

MATERIALS AND METHODS

Chemicals. The following radiolabelled substances were used: (−)-4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]-benzimidazol-2-one with sp. act. 44 Ci/mmol and (−)-[propyl-2,3-^{3}H]-dihydroalprenolol with a sp. act. 38 Ci/mmol, both from Amersham International (Bucks, U.K.). The purity of the radiolabelled ligands was verified by thin layer chromatography (TLC). The following unlabelled compounds were employed: (−)-alprenolol (+)-tartrate from Sigma Chemical Co. (St Louis, MO), (±)-4-(3-t-butylamino-2-hydroxypropoxy)-1,3-dihydrobenzimidazol-2-one hydrochloride from Ciba-Geigy AG (Basle, Switzerland), di-(2-ethylhexyl)-phthalate from Aldrich-Chemie (Steinheim, F.R.G.), tris-(2-butoxyethyl)-phosphate from Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of analytical grade.

Incubation buffer. The medium comprised (mM): NaCl 122, KCL 4.9, $MgSO_4$ 1.2, $CaCl_2$ 1.3 and Na_2HPO_4 15.9, pH 7.38.

AAG. AAG was purified from fresh human serum

* Abbreviations: [^{3}H]-(−)-DHA, (−)-[propyl-2,3-^{3}H]-dihydroalprenolol; [^{3}H]-(−)-CGP 12177, (−)-4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]-benzimidazol-2-one; (±)-CGP 12177, (±)-4-(3-t-butylamino-2-hydroxypropoxy)-1,3-dihydrobenzimidazol-2-one; (−)-ALP, (−)-alprenolol; AAG, α_1-acid glycoprotein; MNL, mononuclear leukocytes; TBEP, tris-(2-butoxyethyl)-phosphate; DEHP, di-(2-ethylhexyl)-phthalate.

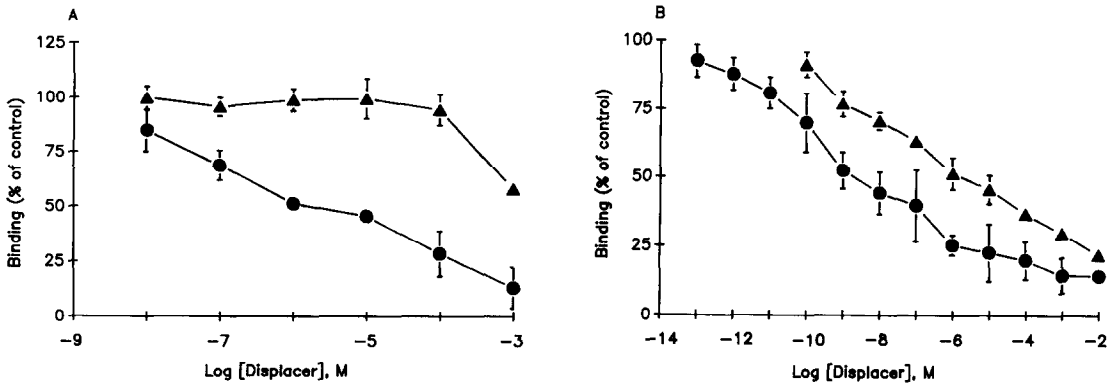

Fig. 1. The effect of increasing concentrations of (−)-ALP, (±)-CGP 12177, TBEP and DEHP on the binding of [^{3}H]-(−)-DHA (3 nM) to AAG (9 μM) at 37°. The binding was determined by equilibrium dialysis. The results are presented as mean value ± SD from five separate experiments. (a) (−)-ALP: ●—●; (±)-CGP 12177: ▲—▲. (b) TBEP: ●—●; DEHP: ▲—▲.

as described previously [6]. The final product appeared homogeneous in SDS–polyacrylamide gel electrophoresis. AAG was dissolved in the incubation buffer before use.

TBEP and DEHP solutions. These substances were emulsified in the incubation buffer by sonication just before the experiments.

Protein binding. The binding of [^{3}H]-(−)-DHA to AAG was determined by equilibrium dialysis using a Dianorm apparatus containing 1 ml Teflon cells. The radioligands and displacers were added to the solution of AAG to obtain a total volume of 900 μl, separated from the buffer (900 μl) by a semi-permeable membrane (Spectrapor membrane tubing, no. 1, M_r cut off 6000–8000, Spectrummedical Industries Inc., Los Angeles, CA). The dialysis was run at 37° in the dark and terminated after 5 hr. Duplicate samples (50 μl) of AAG and buffer were mixed with 3 ml scintillation liquid (Dilusolve, Packard, Groningen, The Netherlands).

Cells. Blood was obtained from healthy young subjects (20–30 years of age). Human mononuclear leukocytes were isolated according to Bøyum [14] using Lymphoprep (Nycomed A/S, Oslo, Norway). MNL were aspirated, washed several times with buffer and stored at 4° for 30 min before experiments. The viability before incubations, determined by the trypan blue exclusion test, was 95–99%. TBEP had a concentration-dependent effect on the MNL trypan blue exclusion. After 30 min in the presence of 0.25 and 250 mM TBEP, the viability was 95–99% and 20–25%, respectively. In contrast, the viability after 1 hr exposure to DEHP, even at the highest concentrations, was not significantly different from control values (95–99%). The extent of binding was related to viable cells only.

Mononuclear leukocyte binding. Intact MNL (2.5–5.0 × 10^6 cells) in a volume of 500 μl, were incubated for 30 min at 37°. Total and non-specific radioligand binding was determined for [^{3}H]-(−)-CGP 12177 concentrations from 0.08 to 2.85 nM in the absence and presence of 0.1 μM (−)-alprenolol, respectively. Total and non-specific radioligand binding was determined for [^{3}H]-(−)-DHA concentrations from 0.1 to 500 nM in the absence or presence of 10 μM (−)-alprenolol. In parallel experiments variable concentrations of TBEP or DEHP were present. The incubations were terminated by addition of 2 ml ice-cold incubation buffer with immediate filtration through a single Whatman GF/C glass-fibre filter which was then washed twice with 10 ml ice-cold buffer. The filtration rate was approximately 1 ml/sec. After ultrafiltration the glass-fibre filters were immediately transferred to counting vials containing 0.5 ml 1 M HCl and 9.5 ml scintillation liquid (Dilusolve, Packard Instr., Groningen, The Netherlands).

Radioactivity. The radioactivity was measured in a Packard Minaxi β-Tri-Carb 4430 Liquid Scintillation Spectrometer.

Calculations and data analysis. Radioligand binding to AAG was calculated from the distribution of the radioactivity between the protein solution and buffer in the dialysis cell. The binding data for MNL were analysed using the IBM-PC version [15] of the software package: "KINETICS, EBDA, LIGAND, LOWRY" (Elsevier Biosoft, Cambridge, U.K.). The program "LIGAND" was originally written by Munson and Rodbard [16].

RESULTS

Displacement of [^{3}H]-(−)-*DHA by* (−)-*ALP,* (±)-*CGP* 12177, *TBEP and DEHP from AAG*

The binding of [^{3}H]-(−)-DHA (3 nM) to purified AAG (9 μM) at 37° was determined in the absence and presence of (−)-ALP (10 nM–1 mM), (±)-CGP 12177 (10 nM–1 mM), TBEP (0.1 pM–10 mM) and DEHP (0.1 nM–10 mM). The bound fraction of [^{3}H]-(−)-DHA in the absence of displacer was 48.4 ± 5.3%, mean ± SD, N = 12. (−)-ALP displaced the radioligand in a concentration-dependent way (Fig. 1a), with an IC_{50} value of 2.01 ± 1.26 μM (mean ± SD, N = 3). (±)-CGP 12177 displaced the radioligand only at the highest concentration tested (1 mM). Separate experiments showed that less than 5% of [^{3}H]-(−)-CGP 12177 (5 nM) was bound to AAG (results now shown).

TBEP and DEHP also displaced [^{3}H]-(−)-DHA

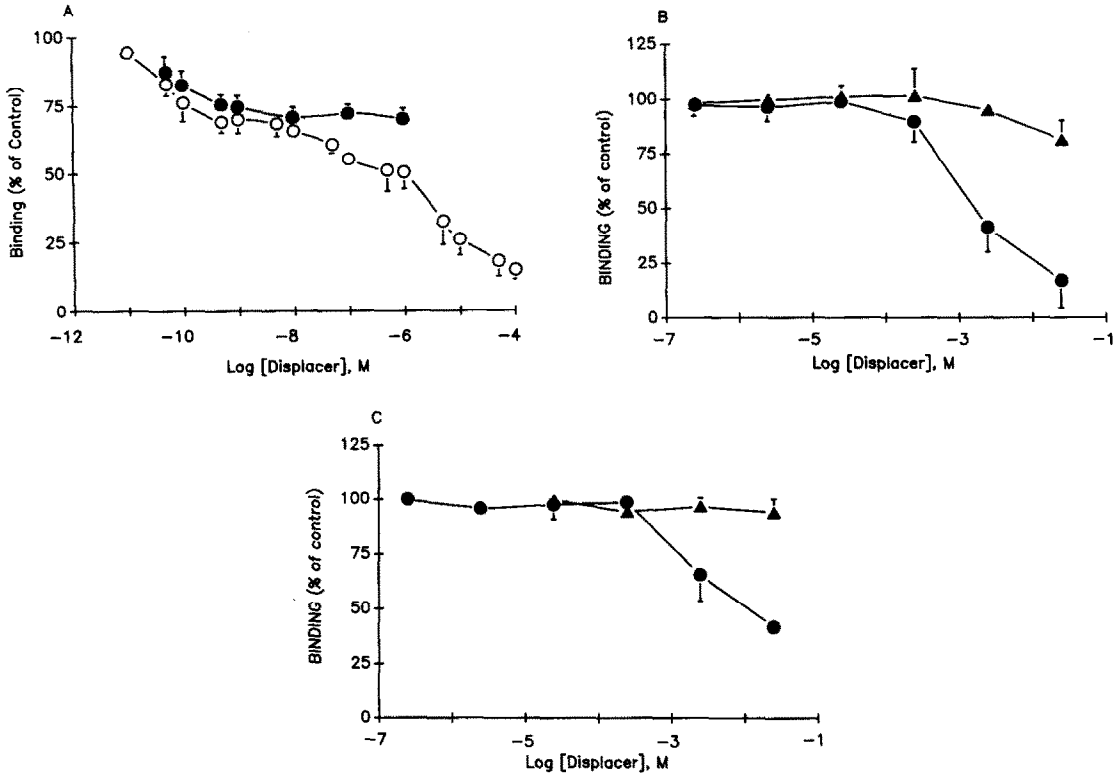

Fig. 2. The effect of increasing concentrations of (−)-ALP, (±)-CGP 12177, TBEP and DEHP on the total binding of [^{3}H]-(−)-DHA (1 nM) or [^{3}H]-(−)-CGP 12177 (0.45 nM) to MNL at 37°. The binding was determined by ultrafiltration. The results are presented as mean value ± SD. (a) Displacement of [^{3}H]-(−)-DHA by (±)-CGP 12177 (●—●) and (−)-ALP (○—○), N = 5. (b) Displacement of [^{3}H]-(−)-DHA by TBEP (●—●) and DEHP (▲—▲), N = 3. (c) Displacement of [^{3}H]-(−)-CGP 12177 by TBEP (●—●) and DEHP (▲—▲), N = 3.

in a concentration-dependent mode from AAG (Fig. 1b). TBEP was the most potent displacer with an IC_{50}-value of 3.53 ± 4.19 nM (mean ± SD, N = 3). The half-maximal displacement of [^{3}H]-(−)-DHA by DEHP occurred at 4.55 ± 3.54 μM (mean ± SD, N = 3).

Displacement of [^{3}H]*-DHA by* (−)*-ALP and* (±)*-CGP* 12177 *from MNL*

The binding of [^{3}H]-(−)-DHA (1 nM) to intact MNL (2.5–5 × 10^6 cells/ml) at 37° was determined in the absence and presence of (−)-ALP (10 pM–0.1 mM) and (±)-CGP 12177 (50 pM–1 μM). (±)-CGP 12177 displaced 25–30% of the total bound radioligand, in a concentration-dependent way (Fig. 2a), with an IC_{50} value of 190 ± 55 pM (mean ± SD, N = 5). Figure 2a shows that (−)-ALP displaced about 90% of the radioligand. However, the displacement occurred in a biphasic manner with a plateau between 1 and 10 nM. The displacement of the high affinity component, representing 30–35% of total bound radioligand, occurred with an apparent IC_{50}-value of 320 ± 130 pM (mean value ± SD, N = 5). The low affinity component, observed for the total radioligand binding between 65–70% and 10–20%, had an apparent IC_{50}-value of 690 ± 210 nM (mean value ± SD, N = 5).

Displacement of [^{3}H]*-(−)-DHA by TBEP and DEHP from MNL*

Figure 2b shows that DEHP was a considerably less potent displacer of [^{3}H]-(−)-DHA (1 nM) bound to MNL than was TBEP. TBEP caused a pronounced and concentration-dependent displacement for concentrations above 10 μM with an apparent IC_{50}-value of 0.94 ± 0.15 mM (mean value ± SD, N = 3). At the highest concentration tested, DEHP displaced about 15% of the bound radioligand.

Displacement of [^{3}H]*-(−)-CGP* 12177 *by TBEP and DEHP from MNL*

The ability to displace [^{3}H]-(−)-CGP 12177 (0.45 nM) bound to MNL was determined for TBEP (250 nM–25 mM) and DEHP (26 μM–26 mM). Figure 2c shows that TBEP causes a concentration-dependent displacement of [^{3}H]-(−)-CGP 12177 binding above 0.1 mM with an apparent IC_{50}-value of 9.4 ± 0.9 mM (mean value ± SD, N = 3). In contrast, DEHP, even at the highest concentration tested, was unable to displace bound [^{3}H]-(−)-CGP 12177 (Fig. 2b).

The effect of TBEP and DEHP on the [^{3}H]*-(−)-CGP* 12177 *saturable and non-saturable binding to MNL*

TBEP (0.25 and 250 mM) and DEHP (2.56 mM)

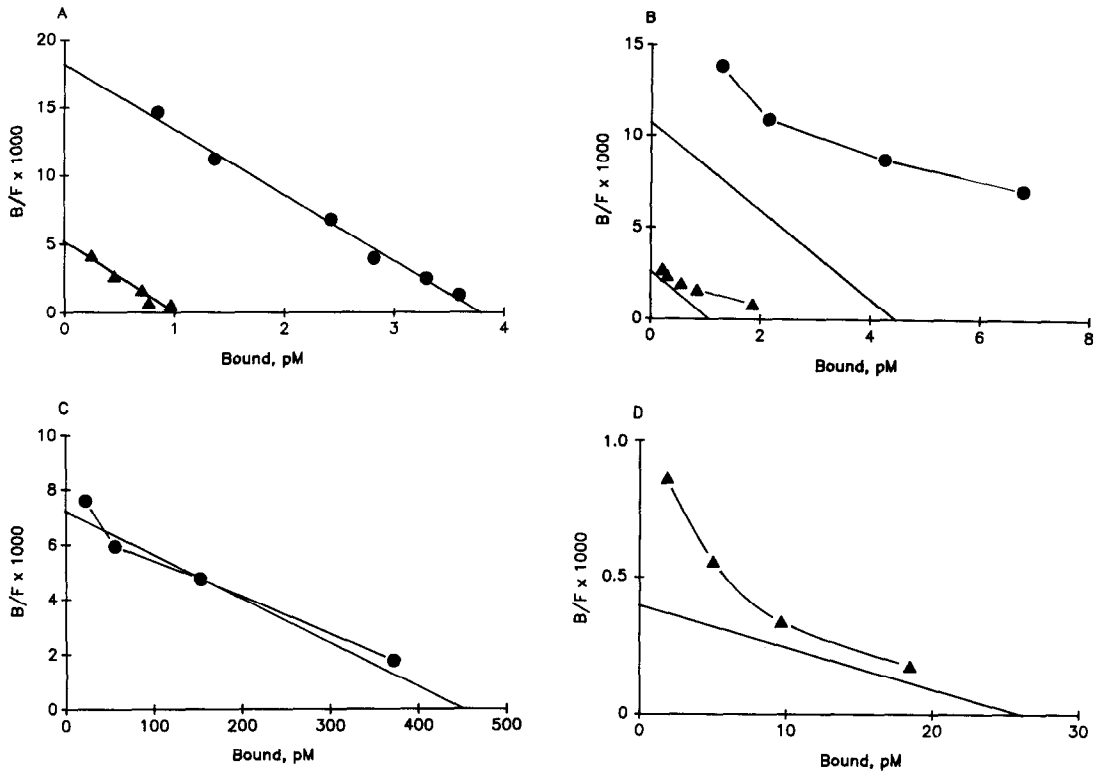

Fig. 3. The effect of TBEP on saturable [^{3}H]-(−)-CGP 12177 and [^{3}H]-(−)-DHA binding to MNL (1×10^6 cells/incubate) at 37°. The binding was determined by ultrafiltration. The results shown are mean values from four separate experiments and are presented as Scatchard plots. The binding lines were obtained using the computer program LIGAND [16]. (a) Saturable [^{3}H]-(−)-CGP 12177 receptor binding in the absence (●—●) and presence of 250 mM TBEP (▲—▲). The straight lines represent receptor-bound radioligand. (b) Saturable [^{3}H]-(−)-DHA binding at low concentrations in the absence (●—●) and presence of 250 mM TBEP (▲—▲). The straight lines represent receptor-bound radioligand. (c) Saturable [^{3}H]-(−)-DHA binding at higher concentrations in the absence of TBEP (●—●). The straight line represents acceptor-bound radioligand. (d) Saturable [^{3}H]-(−)-DHA binding at higher concentrations in the presence of 250 mM TBEP (●—●). The straight line represents acceptor-bound radioligand.

Table 1. Binding parameters for [^{3}H]-(−)-CGP 12177 and [^{3}H]-(−)-DHA bound to MNL β-adrenergic receptors in the absence and presence of TBEP and DEHP results are presented as mean value ± SD)

	[^{3}H]-(−)-CGP 12177		[^{3}H]-(−)-DHA	
Displacer	K_d (pM)	B_{max} (sites/cell)	K_d (pM)	B_{max} (sites/cell)
Series A (N = 3)				
None	180 ± 20	1150 ± 210	420 ± 160	1450 ± 510
TBEP (0.25 mM)	210 ± 40	870 ± 130	410 ± 250	1120 ± 290
Series B (N = 4)				
None	230 ± 50	1280 ± 200	420 ± 140	1350 ± 400
TBEP (250 mM)	260 ± 40	330 ± 30	410 ± 160	330 ± 270
Series C (N = 3)				
None	190 ± 10	1240 ± 390	460 ± 30	1450 ± 430
DEHP (2.6 mM)	210 ± 60	1140 ± 340	490 ± 60	1410 ± 270

were included in radioligand saturation binding experiments to determine whether these substances affected specific and non-specific binding. Figure 3a shows that 250 mM TBEP markedly reduced specifically bound [^{3}H]-(−)-CGP 12177 without affecting the binding affinity. The effect of 0.25 mM TBEP was less pronounced, but clearly demonstrable in all experiments. The number of binding sites was

Table 2. Binding parameters for non-saturable binding of [^{3}H]-(−)-CGP 12177 and [^{3}H]-(−)-DHA to MNL in the absence and presence of TBEP and DEHP (results are presented as mean value ± SD)

Displacer	[^{3}H]-(−)-CGP 12177 (fmol/10^6 cells/nM)	[^{3}H]-(−)-DHA (fmol/10^6 cells/nM)
Series A (N = 3)		
None	0.75 ± 0.08	1.5 ± 0.1
TBEP (0.25 mM)	0.85 ± 0.13	1.0 ± 0.3
Series B (N = 4)		
None	0.73 ± 0.10	1.7 ± 0.5
TBEP (250 mM)	0.65 ± 0.16	1.3 ± 0.4
Series C (N = 3)		
None	0.80 ± 0.15	1.8 ± 0.7
DEHP (2.56 mM)	0.99 ± 0.35	1.7 ± 0.7

Slope (k) of binding line for non-saturable binding given by B (fmol/10^6 cells) = $k * F$ (nM).

reduced whereas the affinity was unaffected (Table 1). Table 2 shows that TBEP had no clear effect on the non-specific binding. The specific and non-specific [^{3}H]-(−)-CGP 12177 binding was not affected by 2.56 mM DEHP (Tables 1 and 2).

The effect of TBEP and DEHP on the [^{3}H]-DHA saturable and non-saturable binding to MNL

Two concentrations of TBEP (0.25 and 250 mM) were tested for their ability to interact with saturable and non-saturable radioligand binding. [^{3}H]-(−)-DHA binds to two different classes of binding sites where only that with highest affinity has the characteristics of β-adrenergic receptors. When the saturable binding was subdivided into its two components, 250 mM TBEP reduced B_{max} of both binding sites without effect on the binding affinities (Fig. 3b–d). Table 1 shows that high-affinity [^{3}H]-(−)-DHA binding was reduced by approximately 25% and 65% in the presence of 0.25 and 250 mM TBEP, respectively. The low-affinity binding was depressed more markedly, by approximately 65% and 90% for 0.25 and 250 mM TBEP, respectively (Table 3). Both TBEP concentrations reduced the non-saturable component by approximately 35% (Table 2).

DEHP (2.56 mM) did not affect the [^{3}H]-(−)-DHA high-affinity binding (Table 1). However, in all experiments a small reduction of the low affinity binding capacity was observed (Table 3). No clear effect was seen for non-specific radioligand binding (Table 2).

Table 3. Binding parameters for saturable low affinity [^{3}H]DHA binding in the absence and presence of TBEP and DEHP (results are presented as mean value ± SD)

	Low-affinity binding	
Displacer	K_d (nM)	B_{max} (sites/cell)
Series A (N = 3)		
None	61 ± 8	144,000 ± 52,000
TBEP (0.25 mM)	74 ± 37	50,000 ± 34,000
Series B (N = 4)		
None	63 ± 22	136,000 ± 45,000
TBEP (250 mM)	65 ± 37	7900 ± 5300
Series C (N = 3)		
None	60 ± 1	134,000 ± 38,000
DEHP (2.56 mM)	60 ± 2	114,000 ± 33,000

DISCUSSION

AAG is the main binding protein for basic drugs in plasma [17]. The present results (Fig. 1a) fit with the previous observations of a close correlation between serum binding of alprenolol and concentrations of AAG [18, 19] and confirmed the reported dissociation constant of about 1 μM for the interaction between alprenolol and AAG [5]. On the other hand, the hydrophilic β-adrenoceptor blocker CGP 12177 had virtually no affinity for AAG. This observation agrees with the finding that mainly lipophilic drugs have affinity for AAG [20, 21]. The plasticizers TBEP and DEHP displace basic drugs from their binding site on AAG [10–12]. TBEP is a more potent displacer than DEHP [11]. In the present study, DEHP had an IC_{50}-value of about 5 μM, similar to the affinity reported for most basic drugs [17]. On the other hand, TBEP caused half-maximal displacement at about 4 nM. This indicates that TBEP has about 1000 times higher affinity for AAG than have most basic drugs.

Ligand binding to cells is often more complex than binding to isolated proteins. Cellular binding may comprise specific binding sites (receptors), non-specific, but saturable binding sites (acceptors) and non-specific/non-saturable binding. Both (−)-ALP and (±)-CGP 12177 reduced the total [^{3}H]-(−)-DHA binding to MNL, but in a different way (Fig. 2a). After a reduction of about 30% of radioligand binding, the (−)-ALP displacement curve reached a plateau at a value similar to the maximal reduction by (±)-CGP 12177. Within this range of concentrations the β-adrenergic receptors become satu-

rated. The further reduction in total [^{3}H]-(−)-DHA binding represents saturation of non-specific/low-affinity binding sites (acceptors). Such MNL acceptor-sites have been previously observed for [^{3}H]-(−)-DHA [1, 8, 9].

Both TBEP and DEHP reduced total [^{3}H]-(−)-DHA binding to MNL (Fig. 2b). As was the case for radioligand binding to AAG, TBEP was much more potent than DEHP. However, the curve pattern did not indicate whether the displacement occurred from receptor or acceptor binding sites. This question was partially answered when [^{3}H]-(−)-CGP 12177 was used as radioligand (Fig. 2c). DEHP had lost most of its displacing ability. This indicated that DEHP mostly has affinity for acceptor rather than receptor binding sites. The effect of TBEP on total [^{3}H]-(−)-CGP 12177 binding, although markedly reduced compared to that on [^{3}H]-(−)-DHA binding, was still present. This showed that both receptor as well as acceptor binding sites represent targets for TBEP.

When TBEP displaced [^{3}H]-(−)-DHA from AAG, a much lower IC_{50}-value was obtained for AAG (≈4 nM) than for MNL (≈1 mM). However, because these plasticizers are extremely lipophilic, they certainly accumulate in the cells and this will result in a large decrease in the extracellular concentration in equilibrium with receptors and acceptors. Because we were unable to determine low concentrations of these plasticizers, the added amounts of TBEP and DEHP were used for calculations. As a consequence, the true IC_{50}-values may be markedly lower. Furthermore, these plasticizers are of very low solubility in water and the true concentrations of properly dissolved material (i.e. monomolecularly dispersed as opposed to droplets and aggregates) are probably much lower than our calculated values.

The effect on receptors, acceptors and non-specific/non-saturable binding was determined by establishing saturation binding curves for [^{3}H]-(−)-CGP 12177 and [^{3}H]-(−)-DHA in the presence of fixed concentrations of plasticizer. TBEP reduced the β-adrenergic receptor binding of both radioligands in a competitive manner (Fig. 3a, b). The difference between the effect of 0.25 and 250 mM TBEP demonstrates a concentration-dependent reduction in β-adrenergic receptors (Table 1). In contrast, DEHP had no effect on radioligand binding to the MNL β-adrenergic receptors (Table 1).

Whereas the β-adrenergic receptor binds both radioligands with high affinity ($K_d \approx 0.2$–0.4 nM), the secondary MNL binding site had no observable affinity for [^{3}H]-(−)-CGP 12177. In concentration saturation experiments, the secondary [^{3}H]-(−)-DHA MNL binding site showed a binding affinity ($K_d \approx 60$ nM) identical to that observed previously [9]. Using the (−)-alprenolol displacement curve (Fig. 2a), the secondary site was found to have a somewhat lower affinity (IC_{50}-value of 0.7 μM), which was very similar to the affinity for alprenolol binding to AAG observed in the present ($K_d \approx 2$ μM) and a previous study ($K_d \approx 0.7$ μM) [5]. This supports the hypothesis that AAG constitutes the low affinity [^{3}H]-(−)-DHA binding sites on MNL [9].

Whereas DEHP (2.56 mM) had no effect on the binding to the β-adrenergic receptor (Table 1), the number of low affinity binding sites was reduced by 15% (Table 3). The effect of TBEP on the acceptors was concentration-dependent. TBEP, at concentrations of 0.25 and 250 mM, reduced the number of low-affinity binding sites by 65% and 95%, respectively (Tables 1 and 3). This shows that the plasticizers have higher affinities for the acceptor than for the receptor binding sites.

In agreement with earlier reports, the non-saturable binding of [^{3}H]-(−)-DHA is higher than that of [^{3}H]-(−)-CGP 12177 (Table 2). Apparently, TBEP and DEHP had no effect on the non-saturable [^{3}H]-(−)-CGP 12177 binding. The apparent ability of TBEP to reduce the non-saturably bound [^{3}H]-(−)-DHA may be explained by incomplete separation between non-specific saturable and non-saturable binding. In saturation binding experiments, the non-saturable binding for [^{3}H]-(−)-DHA, was defined using 10 μM (−)-alprenolol, whereas the (−)-alprenolol displacement curve reached the lower plateau between 0.1 and 1 mM (−)-alprenolol (Fig. 2a).

The observations in the present study may have several pharmacological implications. Drug disposition may be affected by plasticizers. The effect of TBEP and DEHP on AAG binding of basic drugs is well known [10–13] and was discovered as a result of the effect of rubber stoppers of blood sampling tubes [22–24]. It has been shown that blood sampling devices release TBEP leading to *in vitro* concentrations of 0.1–1 mM [13]. The present study confirms the reported effects on AAG and shows, in addition, that these substances may displace drugs from their binding sites in tissues. The pharmacological consequences of such interactions are uncertain, but an increased biologically active fraction may have therapeutic implications. DEHP is released from blood storage bags and patients receiving transfusions obtain high concentrations in blood and tissues [25]. The fact that DEHP affects many hepatic enzymes [26] and dose-dependently increases the effect of pentobarbital [27] suggests that several drugs that utilize hepatic biotransformation mechanisms could have their pharmacological activity affected.

This study shows another effect of plasticizers, previously not recognized, namely the influence on drug and/or hormone signal systems. TBEP may, by its interaction with the β-adrenergic receptor, alter the catecholamine-sensitive adenylate cyclase activity. In contrast, DEHP was virtually unable to displace the radioligands from the β-adrenergic receptors, an observation consistent with the lack of effect of DEHP on the epinephrine response in blood platelets [28].

TBEP, DEHP and other plasticizers represent a toxicological problem because of their wide-spread use in soft plastics and synthetic rubber. This study shows that such substances interact with specific and non-specific binding sites, and may thus have pharmacodynamic as well as pharmacokinetic effects. Further studies are necessary to characterize the toxic potential of plasticizers in relation to drug disposition and effects.

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