ELSEVIER

European Journal of Pharmacology 420 (2001) 97–102

ejp

www.elsevier.nl/locate/ejphar

Pharmacological blockade of brain α_1-adrenoceptors as measured by ex vivo [^{3}H]prazosin binding is correlated with behavioral immobility

Eric A. Stone [a,*], Helen Rosengarten [a], Yan Lin [a], David Quartermain [b]

[a] *Department of Psychiatry TH-HN510, New York University School of Medicine, NYU Med Center, 550 First Avenue, New York, NY 10016, USA*
[b] *Department of Neurology, New York University School of Medicine, 550 First Ave., New York, NY 10016, USA*

Received 21 December 2000; received in revised form 10 April 2001; accepted 18 April 2001

Abstract

The present studies examined the relationship between the blockade of central α_1-adrenoceptors, as measured by ex vivo binding of [^{3}H]prazosin in the cerebral cortex and the inhibition of behavioral activation to a mildly novel environment (cage change). It was found that intraventricular (i.v.t.) terazosin, a saline-soluble α_1-adrenoceptor antagonist, dose dependently inhibited both ex vivo cortical binding and behavioral activation and that there was a highly significant positive correlation between the two with a slope near unity. Prazosin, a nonsaline soluble antagonist which had to be given intraperitoneally (i.p.), was much less potent at blocking both behavioral activity and cortical ex vivo binding, although it blocked ex vivo binding in the lung, indicating that it was effective peripherally but did not readily enter the brain. Despite this, however, the inhibition of cortical binding and behavioral activation that i.p. prazosin did produce were highly correlated with each other and had a slope near unity as with terazosin, whereas the more potent inhibition of lung binding was less well correlated with behavioral inhibition and had a slope significantly less than one. These results confirm our earlier studies, which have shown that α_1-adrenoceptor activity is essential for gross and fine motor behavior in the mouse and that prazosin, which is used extensively in behavioral research, has difficulty entering the mouse brain.

Keywords: α_1-Adrenoceptor; Terazosin; Prazosin; Binding, ex vivo; Exploratory behavior; Motor activity

1. Introduction

It has been known for many years that central α_1-adrenoceptors are a major complement of, or strongly potentiate dopaminergic neurotransmission in the control of motor activity (Anden et al., 1973; Anden and Strömbom, 1974; Segal and Geyer, 1976; Heal, 1984; Plaznik et al., 1984; Pichler and Kobinger, 1985; Plaznik et al., 1985; Snoddy and Tessel, 1985). Recent studies in our laboratory have shown that the activity of these receptors is, in fact, essential for both gross and fine movement in mice and when pharmacologically blocked, results in profound immobility and catalepsy without sedation (Stone et al., 1999, 2001). Our studies were accomplished using intraventicularly administered terazosin and other water soluble α_1-adrenoceptor antagonists, which unlike prazosin, can be dissolved in saline and administered centrally over a wide range of doses. Prazosin, the prototypical α_1-adrenoceptor antagonist, which has been used extensively in behavioral research, is in itself incapable of producing catalepsy when given peripherally (although it can potentiate those produced by dopaminergic antagonists (Pichler and Kobinger, 1985)). This inability may be due to poor penetration of the blood–brain barrier as noted with prazosin in previous studies with mice (Lindroos et al., 1984) and dogs (Taylor et al., 1977). On the other hand, other studies have claimed that prazosin can block central α_1-adrenoceptors in these species at low doses consistent with its high affinity (Pichler and Kobinger, 1985; Mignot et al., 1989). The present experiments were undertaken to resolve this question with regard to prazosin in mice, as well as to shed further light on the relation of brain α_1-adrenoceptors to active behavior. The procedure was to compare the degree of blockade of brain α_1 receptors with the degree of inhibition of behavioral activity. In vivo receptor blockade in the cortex was estimated from the specific ex vivo binding of [^{3}H]prazosin, a method which has been validated in previous studies (Burki, 1986). Lung tissue was used to compare central and peripheral receptor blockade.

* Corresponding author. Tel.: +1-212-263-5740; fax: +1-212-263-0712.
E-mail address: eric.stone@nyu.edu (E.A. Stone).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01003-2

2. Materials and methods

2.1. Subjects

Male Swiss Webster mice, 7–9 weeks old (35–44 g) were used. All animals were singly housed on receipt from the vendor to minimize subordination stress, which may desensitize α_1-adrenoceptors (Stone, 1987). The animals were maintained on a 12 h light/dark cycle (at 0500 h) at 21.1 ± 0.7°C with food and water freely available.

2.2. Iintraventricular (i.v.t.) and intraperitoneal (i.p.) injections

Animals to be used for i.v.t. injection were operated on for implantation of a cannula in the right lateral ventricle by the procedure of Dunn et al. (1987), 5–7 days before the experiment. Terazosin was dissolved in 2 μl of saline and infused in a log series of 10 doses from 0 to 31.6 nmol/mouse at one dose per animal. Details of the i.v.t. injection procedure are described in Stone et al. (2001). Other unoperated animals received i.p. injections of prazosin or vehicle (distilled water) in a log series of seven or eight doses from 0 to 32 mg/kg at one dose per animal, at a volume of 10 ml/kg.

2.3. Behavioral assessment

Two minutes after infusion of terazosin or 1 h after injection of prazosin, animals were placed in a clean "novel" mouse cage ($27 \times 18 \times 6$ cm with 1/8 in Bed O Cobs bedding) of the same type the animals were housed-in (termed the "cage change" paradigm) and videotaped from above for 15 min in the case of the i.v.t. injection or 30 min in the case of the i.p. injection. Behavioral activity was scored manually by a trained observer as the number of gross investigative movements directed at the environment (grooming movements were excluded). These included: (1) locomotion responses to a wall, (2) locomotion of greater than the length of the body while sniffing the bedding, (3) stretch and attend responses and (4) rearing responses (counted separately for each separate spot on the wall or top of the cage that the animal investigated). This scoring method was prompted by the observation that mice exposed to a fresh cage exhibit a number of different types of movements, which all appear to have in common the purpose of investigation of the cage. Individual responses were delimited by a pause, stop, or abrupt change of direction or change of the response itself. All behavioral scoring was made with the videotape playing at fast speed ($5\times$ actual speed), as the segmented sequence of individual acts is more apparent when viewed at this speed. A second trained observer who was blind to the animals' treatments rescored a random sample of 25% of the tapes. Agreement between the two observers was greater than $r = 0.90$ ($df = 38$). The number of gross investigative movements, as measured above, was highly correlated with a more traditional activity scoring method (number of cage quadrants entered, $r = 0.93$, $df = 15$, $p < 0.001$), but was used because it is more sensitive and reflects better the actual organization of the mouse behavior in the novel cage.

2.4. Ex vivo binding

Mice were decapitated immediately after the videotaping, and the cerebral cortices and lung frozen and stored at −80°C. Lung tissue was minced before freezing. Ex vivo [^{3}H]prazosin binding was assayed according to Burki (1986) as follows: tissues were homogenized by Polytron in 50 mM Tris buffer, pH 7.4, for a final concentration of 10 mg wet weight/ml. The crude homogenate was iced for

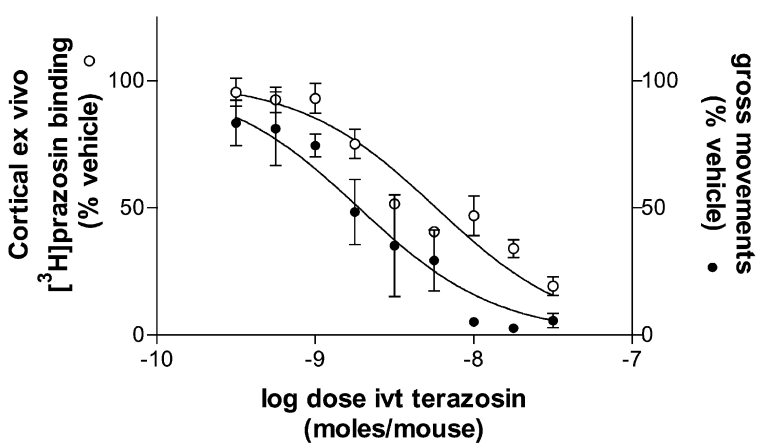

Fig. 1. Dose response curves for the effects of intraventricular terazosin on cortical ex vivo binding of [^{3}H]prazosin at 0.5 nM and on gross investigative movements in same groups of mice. Each point of each curve represents the mean of six mice normalized as a percentage of the mean of seven vehicle-infused control animals that were run simultaneously. Curves were fit by nonlinear regression using a one-site model (see Results). The actual values of the vehicle control were 2.88 ± 0.14 pmoles/g wet weight for ex vivo binding and 170.0 ± 8.4 movements/15 min ($n = 7$) for gross investigative movements.

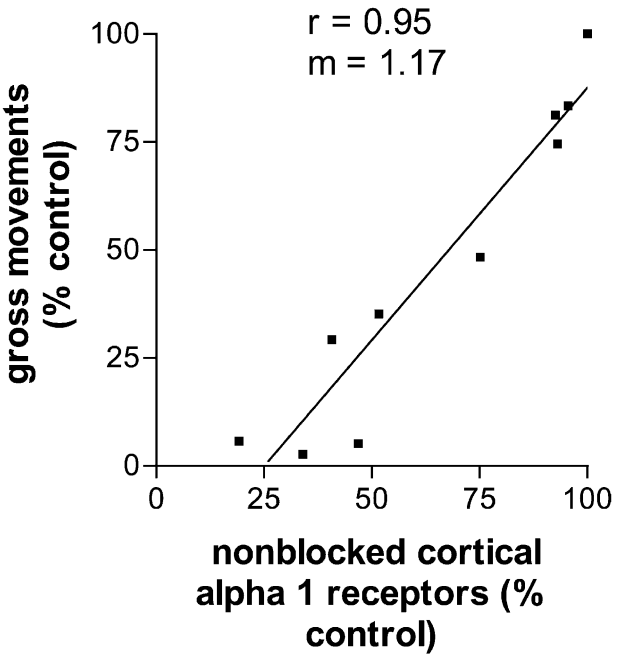

Fig. 2. Scatterplot of the correlation between gross movements and percent of nonblocked cortical α_1-adrenoceptors after i.v.t. terazosin. Each point is the mean gross movements for one dose of the drug. The correlation was significant ($df = 8$, $p < 0.001$).

10–15 min and was then mixed with [^{3}H]prazosin, specific activity 80 Ci/mmol, final concentration 0.5 nM or [^{3}H]rauwolscine, SA 71 Ci/mmol, final concentration 0.75 nM, in the presence or absence of phentolamine, final concentration 10^{-5} M for determination of nonspecific binding. After incubation for 45 min at 27°C, the homogenates were filtered on GF/B filters using a Brandel Cell Harvester and washed 3× with 4 ml 50 mM Tris buffer. The filters were mixed with Liquiscint and counted after 24 h elution.

2.5. Data analysis

The above designs yielded groups of six mice at each dose of each antagonist, with seven control mice given the respective vehicles. For each drug, all of the mean behavioral and binding scores at each dose were normalized as a percentage of the mean of its vehicle control group. Dose–response curves were generated by fitting the normalized data to a one- or two-site competition model by nonlinear regression software (Prism). The program computed the ID_{50} (inhibitory dose 50%) value for each score. Pseudo-Hill plots were generated using the expression, $\log[B/(B-100)]$ where B is the normalized mean binding or behavioral score at a given dose. Slopes of the Pseudo-Hill plots were calculated by linear regression. Product moment correlation coefficients were calculated across the different doses between the behavioral and binding scores with linear regression.

2.6. Chemicals

[7-methoxy-^{3}H]prazosin, SA 80 Ci/mmol and [methyl-^{3}H]rauwolscine, SA 71 Ci/mmol (NEN); terazosin HCl (RBI); prazosin HCl (gift of Pfizer); phentolamine (gift of Ciba).

3. Results

3.1. I.v.t. terazosin

In agreement with our previous study, i.v.t. terazosin produced a dose-dependent sigmoidal complete inhibition of gross movements (Fig. 1). This was adequately fit to a one-site competition curve ($R^2 = 0.97$) with no improvement in fit by a two-site model. The ID_{50} (95% confidence

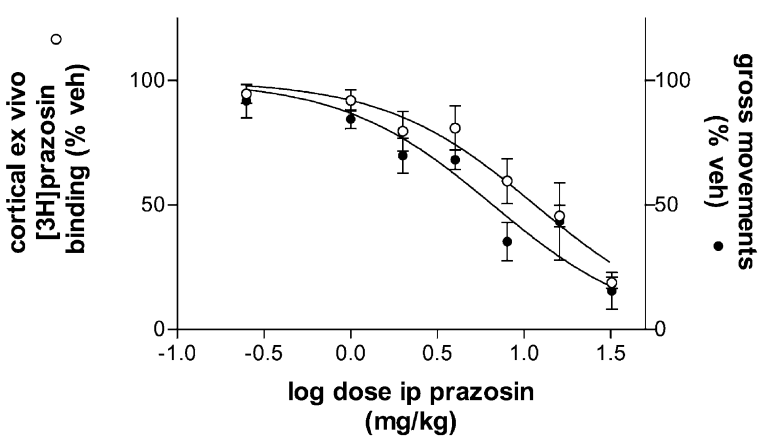

Fig. 3. Dose response curves of effect of i.p. prazosin on ex vivo cortical binding and gross movements (see legend to Fig. 1). Each point represents the mean of six mice. Actual values of vehicle controls were 3.19 ± 0.31 pmoles/g wet weight cortex for ex vivo binding and 372 ± 27 movements/30 min for gross investigative movements ($n = 6$).

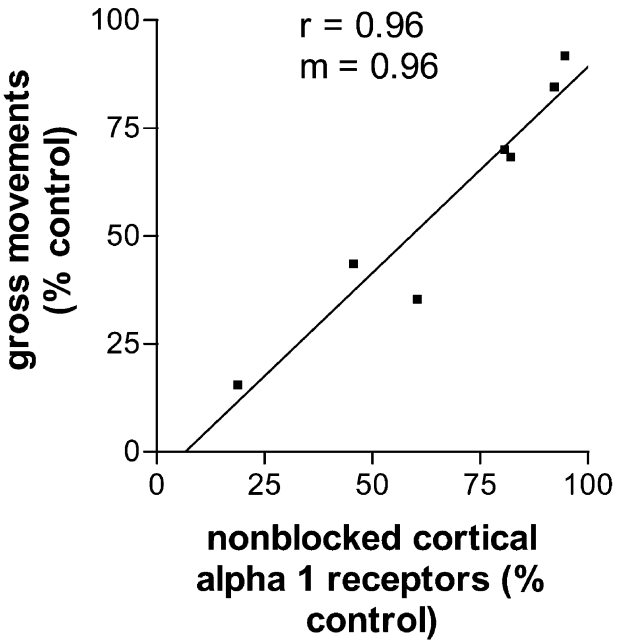

Fig. 4. Scatterplot of the correlation between gross movements and percent of nonblocked cortical α_1-adrenoceptors after i.p. prazosin. Each point is the mean movement for one dose of prazosin. The correlation was significant ($df = 5$, $p < 0.005$).

interval) was 1.88 (1.44–2.47) nmol/mouse. A Pseudo-Hill plot of the means revealed an n_H not significantly different from unity (1.20 ± 0.13) (not shown).

In the same animals, terazosin produced a dose-dependent reduction of ex vivo specific [^{3}H]prazosin binding in the cerebral cortex (Fig. 1). The data were adequately fit to a one-site model ($R^2 = 0.92$) with no improvement by a two-site model. The Pseudo-Hill plot revealed an n_H not significantly different from unity (1.00 ± 0.10) (not shown). The ID_{50} was 5.79 (4.04–8.30) nmol/mouse, which was significantly greater than the above ID_{50} for behavioral inhibition. There was a highly significant positive correlation of mean behavioral inhibition and mean binding inhibition across the dosages (Fig. 2). For the purpose of the correlation, the mean binding values were subtracted from 100% and expressed as percent nonblocked receptors. The slope of the regression line was not significantly different from unity (1.17 ± 0.13). Total inhibition of behavioral activity occurred at significantly above zero nonblocked α_1-adrenoceptors sites (25.0% (9.1–35.4%)). The regression line did not deviate significantly from linearity by the runs test. At the highest dose used (31.6 nmol/mouse), terazosin did not affect ex vivo 0.75 nM [^{3}H]rauwolscine binding in the cortex (vehicle, 3.72 ± 0.36; terazosin, 5.09 ± 0.72 pmol/g wet weight, NS).

3.2. I.P. prazosin

Peripherally administered prazosin also led to a dose-dependent inhibition of active behavior and ex vivo cortical [^{3}H]prazosin binding (Fig. 3). Both curves were adequately fit to a one-site competition model (R^2s: behavior, 0.91; binding, 0.97). As in the case of terazosin, the ID_{50} for the behavioral inhibition, 6.59 (4.30–10.10) mg/kg, tended to be smaller than that of the binding inhibition, 11.55 (8.79–15.18) mg/kg, but this was not statistically significant. Again, as in the case of i.v.t. terazosin, there was a highly significant positive correlation between the inhibitions of binding and behavior, with the slope of the regression line not significantly different from unity (Fig. 4). The regression line did not deviate significantly from linearity by the runs test.

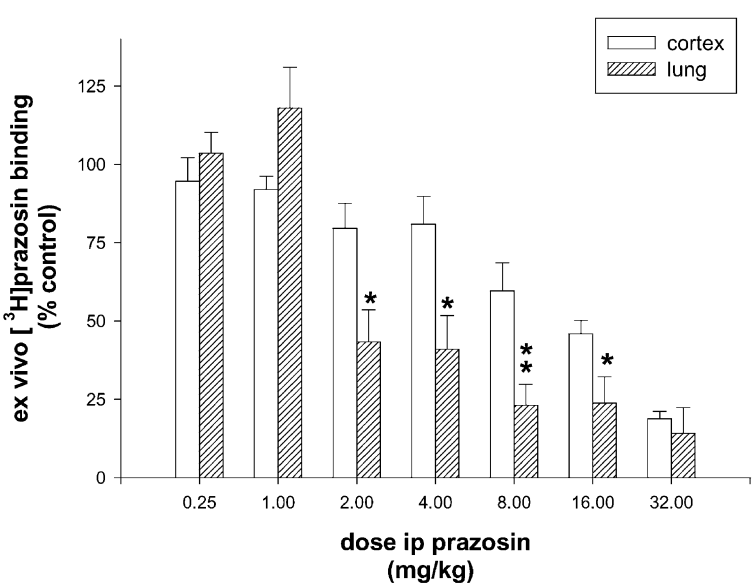

Fig. 5. Comparison of ex vivo [^{3}H]prazosin binding in the cerebral cortex and lung after i.p. prazosin. Both tissues were obtained from the same animals. $N = 6$ per group. Actual value of lung binding of control group was 12.5 ± 1.2 pmoles/g wet weight. $^{*}p < 0.05$, $^{**}p < 0.01$ t-test.

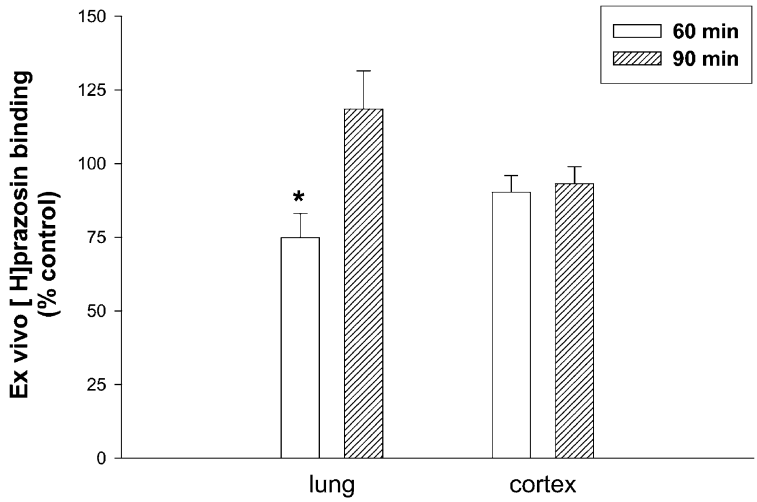

Fig. 6. Comparison of ex vivo binding in the lung and the cortex at two times after i.p. injection of 1 mg/kg prazosin. $N = 6$ per group. * $p < 0.02$, t-test.

I.P. prazosin was slightly more potent in inhibiting ex vivo binding in the lung, as compared to the cortex (ID_{50}, 3.68 (1.33–10.15) mg/kg; cortex, see above, $p < 0.05$) but no difference in potency was found for lung binding inhibition compared with behavioral inhibition (see above). However, the drug had a significantly greater efficacy in inhibiting lung binding than cortical binding at the 2, 4, 8 and 16 mg/kg doses (Fig. 5). Moreover, if the interval between the injection and sacrifice was shortened from 90 to 60 min, the 1 mg/kg prazosin dose became effective for the lung but remained ineffective for the cortex (Fig. 6). There was a significant positive correlation between inhibition of lung binding and inhibition of behavioral activity; however, the slope of the regression line was only 0.59 ± 0.15, which is significantly less than unity ($p < 0.01$) indicating a weaker relationship than that between cortical binding and behavioral inhibition (Fig. 7).

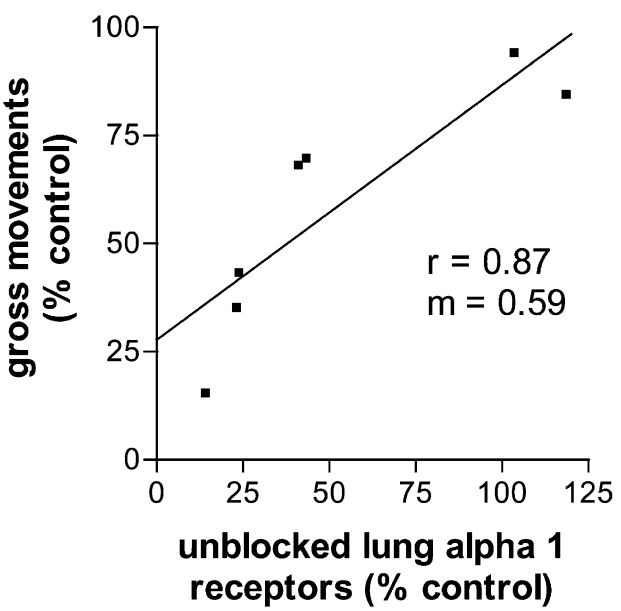

Fig. 7. Scatterplot of the correlation between gross movements and percent of unblocked lung receptors. For details, see legend to Fig. 2. The correlation was significant ($df = 4$, $p < 0.05$), however, the slope of the regression line was significantly less than unity (see text), indicating a weak relationship.

4. Discussion

The present experiments show that there is a high correlation between the pharmacological blockade of central α_1-adrenoceptors, as measured by ex vivo binding of [^{3}H]prazosin in the cerebral cortex and the inhibition of behavioral activity in a mildly novel environment. This correlation held for both terazosin, a saline soluble antagonist, which was given centrally, and for prazosin, which is not saline soluble and had to be given i.p. The highest dose of i.v.t. terazosin used (31.6 nmol/mouse) did not inhibit cortical ex vivo α_2-adrenoceptor binding as measured by [^{3}H]rauwolscine, indicating that terazosin maintained its selectivity for α_1 receptors under these conditions. The results confirm our previous findings with a series of i.v.t. α_1-adrenoceptor antagonists cited above, which have shown that the activity of α_1-adrenoceptors of the 1B subtype is essential for both gross and fine movement in the mouse. They also confirm a much earlier study, which showed that there is a positive correlation between receptor occupancy of an α_1-adrenoceptor agonist in the spinal cord as measured by ex vivo [^{3}H]prazosin binding and the potentiation of startle responses (Astrachan et al., 1983).

Although there was a high correlation between inhibition of binding and inhibition of behavior, the apparent affinity of i.v.t. terazosin for behavioral inhibition was greater than for binding inhibition. This effect was less apparent with i.p. prazosin. It may be argued that this difference was due to some dissociation of the cold antagonist during the homogenization step. However, this is unlikely since Burki (1986), whose method we employed, has shown that the specific ex vivo binding of [^{3}H]prazosin

to brain α_1-adrenoceptors does not decline at 4°C until the third homogenization and centrifugation. An alternative explanation for the above difference is that since the animals were killed after the behavioral test, there was significant in vivo washout of terazosin from brain receptors prior to sacrifice. This is supported by the finding that there was a significant reduction in inhibition of cortical binding in 30 min of washout between 60 and 90 min post-prazosin injection. Furthermore, a washout effect would be expected to be greater with i.v.t. than i.p. injection, since the latter would be buffered by the pool of antagonist in the body.

The above findings also suggest why prazosin given peripherally does not produce the marked immobility and catalepsy seen after i.v.t. administration of saline soluble antagonists. Prazosin was found to block a significantly greater percentage of peripheral (as measured by lung) than central (as measured by cerebral cortical) receptors at lower dosages, indicating that the drug has difficulty entering the brains of these animals. Moreover, using a range of dosages over which prazosin enters the brain (up to 32 mg/kg), a virtually identical correlation was found between inhibition of behavior and binding with i.p. prazosin and i.v.t. terazosin, suggesting that the two drugs were acting on the same population of α_1-adrenoceptors. It is unlikely that the higher blood flow to the lungs vs. brain was the cause of the greater binding inhibition in the former tissue, since this would not be expected to alter the concentration of the antagonist. Therefore, these findings confirm for the present mouse strain the previous studies cited in the Introduction, which have found difficulty in the entry of prazosin in the mouse and dog brain. Whether this is applicable to the rat remains to be tested in future research.

Although i.p. prazosin was clearly more effective in the lung than the cortex, the drug still appeared not very efficient in blocking lung receptors until very large doses (8–16 mg/kg) were given. Since we used a fairly long interval between i.p. injection of the drug and sacrifice (90 min), in order to provide sufficient time for brain penetration, this low efficacy was probably an artifact of the washout of prazosin from peripheral tissues prior to sacrifice (Cavero and Roach, 1980). In confirmation, as noted above, there was a significant washout of prazosin from the brain between 60 and 90 min post-injection.

Acknowledgements

This research was supported by an NIMH grant MH45265. The authors thank Dr. Kenneth Minneman for his helpful advice and consultation.

References

Anden, N.E., Strömbom, U., 1974. Adrenergic receptor blocking agents: effects on central noradrenaline and dopamine receptors and on motor activity. Psychopharmacologia 38, 91–103.

Anden, N.E., Strömbom, U., Svensson, T.H., 1973. Dopamine and noradrenaline receptor stimulation: reversal of reserpine-induced suppression of motor activity. Psychopharmacologia 29, 289–298.

Astrachan, D., Davis, M., Gallager, D., 1983. Behavior and binding: correlations between α_1-adrenergic stimulation of acoustic startle and α_1-adrenoceptor occupancy and number in rat lumbar spinal cord. Brain Res. 260, 81–90.

Burki, H.R., 1986. Binding of psychoactive drugs to rat brain amine receptors, measured ex vivo, and their effects on the metabolism of biogenic amines. Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 258–266.

Cavero, I., Roach, A., 1980. The pharmacology of Prazosin, a novel antihypertensive agent. Life Sci. 27, 1525–1540.

Dunn, A.J., Berridge, C.W., Lai, Y.I., Yachabach, T.L., 1987. CRF-induced excessive grooming behavior in rats and mice. Peptides 8, 841–844.

Heal, D.J., 1984. Phenylephrine-induced activity in mice as a model of central α_1-adrenoceptor function. Effects of acute and repeated administration of antidepressant drugs and electroconvulsive shock. Neuropharmacology 23, 1241–1251.

Lindroos, O., Leinonen, L., Hilakivi, I., 1984. Autoradiographic picture of mouse brain after intraperitoneal injection of [^{3}H]prazosin. Acta Univ. Tampere 21, 131–141.

Mignot, E., Guilleminault, C., Bowersox, S., Frusthofer, B., Nishino, S., Maddaluno, J., Ciaranello, R., Dement, W.C., 1989. Central α_1-adrenoceptor subtypes in narcolepsy–cataplexy: a disorder of REM sleep. Brain Res. 490, 186–191.

Pichler, L., Kobinger, W., 1985. Possible function of α_1-adrenoceptors in the CNS in anesthetized and conscious animals. Eur. J. Pharmacol. 107, 305–311.

Plaznik, A., Danysz, W., Kostowski, W., 1984. Behavioral evidence for α_1-adrenoceptor up and α_2-adrenoceptor down regulation in the rat hippocampus after chronic desipramine treatment. Eur. J. Pharmacol. 101, 305–306.

Plaznik, A., Danysz, W., Kostowski, W., 1985. A stimulatory effect of intraaccumbens injections of noradrenaline on the behavior of rats in the forced swim test. Pol. J. Pharmacol. 87, 119–123.

Segal, D.S., Geyer, M.A., 1976. Pre- and post-junctional supersensitivity: differentiation by intraventricular infusions of norepinephrine and methoxamine. Psychopharmacology 50, 145–148.

Snoddy, A.M., Tessel, R.E., 1985. Prazosin: effect on psychomotor-stimulant cues and locomotor activity in mice. Eur. J. Pharmacol. 116, 221–228.

Stone, E.A., 1987. Central cyclic AMP-linked noradrenergic receptors: new findings on properties as related to the actions of stress. Neurosci. Biobehav. Rev. 11, 391–398.

Stone, E.A., Zhang, Y., Rosengarten, H., Yeretsian, J., Quartermain, D., 1999. Brain alpha 1-adrenergic neurotransmission is necessary for behavioral activation to environmental change in mice. Neuroscience 94, 1245–1252.

Stone, E., Lin, Y., Itteera, A., Quartermain, D., 2001. Pharmacological evidence for the role of brain alpha 1B-adrenergic receptors in the motor activity and spontaneous movement of mice. Neuropharmacology 40, 254–261.

Taylor, J.A., Twomey, T.M., Schach von Wittenau, M., 1977. The metabolic fate of prazosin. Xenobiotica 7, 357.