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Effects of Propranolol on, and Noradrenergic Correlates of, the Response to Nonreward

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MARSLAND, A. L., P. SALMON, P. TERRY AND S. C. STANFORD. *Effects of propranolol on, and noradrenergic correlates of, the response to nonreward.* PHARMACOL BIOCHEM BEHAV **35**(1) 41–46, 1990.—Rats were rewarded by food for running in a straight runway with short (15 sec) intertrial intervals. On the final day, animals were subjected to either 14 extinction trials or 14 rewarded trials. During acquisition, half of each group had been injected once daily for 15 days with propranolol (5 mg/kg IP), the remainder with saline vehicle. All animals were killed immediately after the final trial and the cerebral cortex taken for noradrenaline assay and radioligand binding to beta- and alpha$_2$-adrenoceptors. Propranolol increased running times early in extinction; this effect was replicated in a second experiment. Neither the drug injections nor the extinction procedure affected neurochemical measures. However, the rate of extinction correlated positively with both beta- and alpha$_2$-adrenoceptor number. Although consistent with the theory that beta-adrenoceptors are involved in adaptation to stress, these results differ from our previous findings. The relationship between beta-adrenoceptor number and the response to stress may depend on the severity of the stress.

Adrenoceptors Massed extinction Noradrenaline Nonreward Propranolol Rat Stress

IT is widely held that beta-adrenoceptor desensitization is one neurochemical component of adaptation to stress (5, 15, 17). Specifically, a decrease in beta-adrenoceptor number and/or coupling to the cyclic AMP second messenger system is thought to underlie the reduction in adverse behavioural responses to repeated stress. In recent experiments with rats we examined this theory by measuring the correlation between individual animals' adrenoceptor number and their behavioural response to mild forms of psychological stress: placement in a novel open field (10), and frustration administered by the extinction of continuously reinforced running (12). From the theory, it would be predicted that the lower the number of receptors, the more a rat will resist the stress. Contrary to this prediction we found that the lower the number of cerebral cortical beta-adrenoceptors, the less the animal moved towards the centre of the open field or the less it persisted running in extinction; i.e., the *less* resistant it was to the stress. Resistance to nonreward also correlated positively with alpha$_2$-adrenoceptor density.

There are two possible explanations for the discrepancy between these findings and the theory from which the predictions were drawn: the first is that the relationship between beta-adrenoceptors and behaviour differs between mild stress, such as nonreward or the open field, and the more severe forms which are generally used in this sort of work (e.g., immobilization and electric shock). The second is that the neurochemical 'coding' of individual differences in response to stress may differ from that underlying adaptation to stress; that is, different neurochemical processes might determine 'trait' and 'state' aspects of the response to stress.

The present experiments addressed both of these alternatives. Once again, we examined the correlation between beta-adrenoceptor density and the response to nonreward, but this was administered in a different way from that in our previous study (12). Whereas, in that case, nonrewarded trials were 24 hours apart, they were separated by only 15 sec in the present experiments. Since this faster procedure ensures that extinction is completed within about 30 minutes, it might be regarded as producing a more intense form of stress. There are, of course, other unavoidable differences between the two forms of stress, notably its duration. Nevertheless, the type of stressor in the two cases is unchanged.

We also investigated whether differences in behaviour caused by neurochemical adaptation ('state') are related to receptor density in the same way as basal individual differences ('trait'). To do this, we administered the beta-adrenergic blocker, propranolol, chronically to one group of animals. Findings that repeated admin-

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istration of propranolol upregulates beta-adrenoceptor number (2,3) and accelerates the inhibition of nonrewarded responses (9) support the view that beta-adrenoceptor desensitization is associated with adaptation to stress. Two rewarded control groups (one saline and one drug) were also included to confirm both that behavioral effects of propranolol were restricted to nonrewarded running and that the extinction procedure did not itself influence adrenoceptor binding. To establish whether the behavioural effect of propranolol in this paradigm was robust, it was replicated in a second experiment.

Finally, we measured two further neurochemical parameters: radioligand binding to alpha$_2$-adrenoceptors, because of evidence for their involvement in adaptation to mild stress (12,13), and cortical noradrenaline concentration. Although a crude measure of changes in the functional state of transmitter stores, noradrenaline is involved in adrenoceptor regulation and, in previous experiments, its concentration correlated with the number of alpha$_2$-adrenoceptors (12).

METHOD

Subjects

Naive male Sprague-Dawley rats (42, Experiment 1; 20, Experiment 2) from OLAC (Bicester, UK) were housed in groups of 5 or 6 with water freely available. Food was freely available for 2 weeks, by which time mean weight had reached 292 and 258 g in Experiments 1 and 2, respectively. Thereafter, animals were fed daily (15 g/rat) in their home cage after behavioural testing was complete. Testing was from the 2nd–5th hours of the light phase of a 12/12-hour light/dark cycle.

Apparatus

A straight runway, 32 cm high × 15 cm wide, of black perspex, was divided into start, run and goal sections (20, 110 and 20 cm long, respectively) by remotely operated doors. The end wall of the goal-box contained a food magazine into which 5 × 45 mg reward pellets (Noyes) could be placed as reward. Latency to leave the start box was measured from opening the door to breaking a photobeam 3.5 cm into the run section, Run time from this to its breaking a beam 3.5 cm before the goal-box door, and latency to take the reward from this to breaking a beam mounted immediately in front of the food magazine. Run time is used as the principal measure, unaffected by the time taken to emerge from the start-box or to enter the food magazine. For comparison, Total time, from the opening of the start-box door to the breaking of the final beam, is also reported. Times were recorded and doors operated by an Acorn microcomputer programmed in Onlibasic. The alley was dimly illuminated by a 60-W bulb directed at the ceiling above. Rats were confined singly in waiting boxes before and after each exposure to the alley, which was cleaned with a damp cloth, and then wiped dry, after each animal had completed its daily series of trials (see below).

Behavioural Training

After preliminary handling (7 days, Experiment 1; 10 days, Experiment 2) rats were familiarized with the alley over 2 days. On the first they were allowed to explore in cage groups for 15 min; on the second, individually for 10 min. On both days, reward pellets were scattered throughout the alley.

Acquisition training began on the following day. Reward was placed in the food magazine before each trial. The start-box door was opened 5 sec after the rat was placed in the start-box. The number of daily trials was gradually increased from 1 to 10 (Experiment 1) or 7 (Experiment 2); total trials were 75 over 15 days (Experiment 1) and 45 over 12 days (Experiment 2). From the 8th trial, the goal-box door was closed immediately the final photobeam was broken, confining the rat for 15 sec. Animals were replaced in the start-box immediately after removal from the previous trial. One rat was eliminated from Experiment 2 because of inconsistent running.

On the following day, two groups of animals in Experiment 1 (see below) received 14 further acquisition trials. For the remainder in this experiment, and for all animals on the final day of Experiment 2, running was extinguished (over 14 or 16 trials in Experiments 1 and 2, respectively). If Run time, latency to leave the start-box, or latency to take food reached 60 sec (Experiment 1) or 100 sec (Experiment 2), the animal was removed and credited with this time for that and subsequent stages of the alley. If this occurred on two consecutive trials, it was deemed to have extinguished and credited with this time for each measure on subsequent trials.

Drug Testing

Experiment 1. Before acquisition training, animals were divided into 4 groups matched for mean weight. The Drug group was injected IP with racemic propranolol HCl (5 mg/kg) dissolved in isotonic saline (1 ml/kg) 1 hour after each day's final acquisition trial. Fifteen injections were given in total; this duration of treatment is in excess of that reported to cause upregulation of beta-adrenoceptor number (2). The Saline group received vehicle at these times. These groups were further subdivided. Groups Drug-Extinction and Saline-Extinction underwent extinction trials on the final day; groups Drug-Reward and Saline-Reward underwent further acquisition trials. One Saline-Rewarded rat escaped before final testing and was eliminated from the experiment. Mean weights at the start of acquisition were Drug-Extinction (N = 11): 301 g; Drug-Reward (N = 10): 300 g; Saline-Extinction (N = 11): 301 g; Saline-Reward (N = 9): 300 g.

Experiment 2. Animals were divided into 2 groups, matched for mean weight and for mean running time over acquisition days 3–6. Fifteen minutes after an animal's final trial on each subsequent acquisition day, it was injected IP. Six injections were given in total. The Drug group (n = 9; mean weight, 270 g) received racemic propranolol as above; the Saline group (N = 10; mean weight, 272 g) received vehicle alone. In a previous study this drug regime was sufficient to produce a disinhibitory effect on nonrewarded responses (9).

Membrane Preparation and Radioligand Binding (Experiment 1)

Immediately after the final extinction trial, animals were taken individually to an adjacent room and killed by cardio-thoracic shock and cervical dislocation. The cerebral cortices were dissected on ice and stored at −18°C. Immediately before binding, the cortices were chopped and half the tissue taken for radioligand binding; the remainder was used for analysis of noradrenaline content (see below). Tissues for radioligand binding were homogenised in 8 ml Tris buffer (pH 7.7, 50 mmol) and centrifuged at 20000 × g for 20 min. The pellet was washed (in 2 × 2 ml Tris buffer), resuspended in 8 ml Tris buffer and recentrifuged at 20000 × g for 20 min. The final pellet was again washed (in 2 × 2 ml Tris buffer) and resuspended to given an equivalent of 20 mg original wet weight of tissue per ml of Tris buffer. As far as possible, all binding assays contained one sample from each treatment group, and the sequence of samples in successive assays was balanced in a Latin square. The binding assay used [^{3}H]dihydroalprenolol as the radioligand for the beta-adrenoceptor

binding sites and [^{3}H]clonidine to bind to alpha$_2$-adrenoceptor binding sites. Six concentrations of each radioligand were used, ranging from 0.2 to 4 nM. Nonspecific binding was assessed by coincubation of membranes and radioligands with 10 μM d,l-propranolol (beta) or 10 μM phentolamine (alpha$_2$). Specific binding accounted for approximately 70% of total [^{3}H]dihydroalprenalol bound and 85% of [^{3}H]clonidine. All samples were incubated in duplicate for 40 min at 26°C in a total volume of 250 μl of buffer. Incubation was terminated by filtration using a membrane harvester. Filters were rapidly washed with 7 ml Tris buffer.

Radioligand binding was assessed by liquid scintillation counting and was analysed using a nonlinear regression, iterative, computer program [(8), modified by Jackson and Edwards, unpublished, UCL]. The number of receptors (B_{max}) was expressed in terms of the protein content of the samples (6); the binding dissociation constant (K_d) is expressed in terms of radioligand concentration, nM.

Measurement of Noradrenaline (Experiment 1)

Tissues were weighed and homogenized in 10 volumes of 0.1 M $HClO_4$ and centrifuged to remove the protein precipitate. Noradrenaline in the supernatant was separated by HPLC, using a citrate/acetate buffered mobile phase pH 5.8 containing 10% methanol and 600 mg/l octane sulphonic acid. The content was measured by electrochemical detection and noradrenaline concentration expressed in terms of wet weight of tissue.

Statistical Methods

Behaviour (Experiments 1 and 2). Running times for the final day of acquisition and for the extinction day were $\log_{10}$-transformed to normalize their distributions and subjected to analyses of variance. The within-subject term was Trials. Between-subjects terms were Drug and, in Experiment 1, Extinction (distinguishing groups undergoing extinction from those receiving further rewarded trials). A polynomial expansion to cubic trend was fitted to Trials; trends were assessed a priori (4) since we were concerned with changes in behaviour which develop gradually through extinction. Where the F-ratio for an interaction was significant, specific comparisons were tested by *t*-tests, using the error term from the analysis of variance. Similar analyses were performed on running times on the final two acquisition days.

Neurochemistry (Experiment 1). Neurochemical parameters were $\log_{10}$ transformed to normalize distributions. Each was then subjected to a regression analysis. This removed effects of assay batch before testing those of the two factors, Drug and Extinction, and their interaction.

Behaviour and neurochemistry (Experiment 1). Within-group product-moment correlations were calculated between noradrenaline and binding measures for the rewarded and extinguished animals separately, and between all neurochemical measures and three behavioural indices in the animals undergoing extinction: mean running time in extinction, running time on the first extinction trial, and the slope of the regression of running time on trial (the index of speed of extinction). For the animals which continued to be rewarded, correlations were calculated with the mean running time during the final 14 trials.

RESULTS

Behaviour

Experiment 1. The drug groups did not differ during acquisition; mean running times over the final acquisition day are virtually identical (Fig. 1). As expected, the extinction procedure gradually increased Run times over trials [Fig. 1; Extinction: $F(1,37)=25.54$, $p<0.001$; Extinction × Linear trend of Trial: $F(1,481)=29.84$, $p<0.001$]. Evidence for a drug effect was seen in the interaction of Drug × Extinction × Cubic trend of Trial, $F(1,481)=7.10$, $p<0.01$. Inspecting the coefficients for each group showed that this effect was attributable to the comparison between the Propranolol-Extinction and Saline-Extinction groups ($t=3.3$, $p<0.01$; values for Propranolol-Extinction, Saline-Extinction, Propranolol-Reward, Saline-Reward: 0.0011, −0.0012, −0.0003, 0.0000, respectively; S.E.: 0.0005). This confirms the effect seen in Fig. 1 whereby running times lengthened in the Drug group more rapidly than in Saline, and subsequently stabilized before appearing to exceed Saline again by the end of extinction testing. Drug had no effect in rewarded groups. A similar pattern of differences was seen in Total times, in which the interaction of Drug × Extinction × Cubic trend of Trial approached significance, $F(1,481)=2.88$, $p=0.09$.

Experiment 2. Figure 1 shows a drug effect which is similar in essential respects to that seen in Experiment 1. Run times lengthened more rapidly in Drug animals over intermediate trials, but then appeared to shorten before exceeding Saline again by the end of testing. Once again, this was confirmed in the interaction of Drug × Cubic trend of Trials, $F(1,254)=8.71$, $p<0.01$. The cubic coefficient differed significantly from zero in the drug group, but not saline (values for drug and saline groups: 0.0011, −0.0006, respectively; S.E. 0.00041). The Drug × Trial interaction also reached significance, $F(15,254)=2.38$, $p<0.01$. A similar pattern was seen in Total time, in which the interaction of Drug × Cubic trend of Trial approached significance, $F(1,254)=3.68$, $p=0.06$.

Behaviour and Neurochemistry (Experiment 1)

Correlations. Noradrenaline concentration correlated with alpha$_2$-adrenoceptor K_d ($r=.53$, $p<0.05$), but not B_{max} ($r=.37$, $p<0.10$). There was no relationship between noradrenaline concentration and beta-adrenoceptor B_{max} or K_d ($rs=.05, .15$, respectively).

Correlations with the rate of extinction are summarized in Table 1, and the significant ones are portrayed in Fig. 2. Density (indexed by B_{max}) of both alpha$_2$- and beta-adrenoceptors correlated positively with the rate at which running times lengthened over trials in the animals undergoing extinction. Receptor affinity (indexed by K_d) and noradrenaline concentration were unrelated to rate of extinction. There were only isolated correlations with mean running times over the final 14 trials in each group: Run times correlated negatively with beta-adrenoceptor B_{max} ($r=-.49$, $p<0.05$) in the rewarded animals, and with noradrenaline concentration in the extinguished animals ($r=-.50$, $p<0.05$). Although noradrenaline correlated significantly with each, the correlation between alpha$_2$-adrenoceptor K_d and mean Run time in extinction did not approach significance ($r=-.36$, $p>0.1$). There were no correlations with running time on the first extinction trial.

Regression analyses. Groups did not differ on any neurochemical measure.

DISCUSSION

In our previous experiment, we found that numbers of both beta- and alpha$_2$-adrenoceptors correlated negatively with the rate of extinction of running (12). In that experiment, 10 extinction trials were carried out at the rate of 1/day. In the present experiment, we have investigated whether the relationship be-

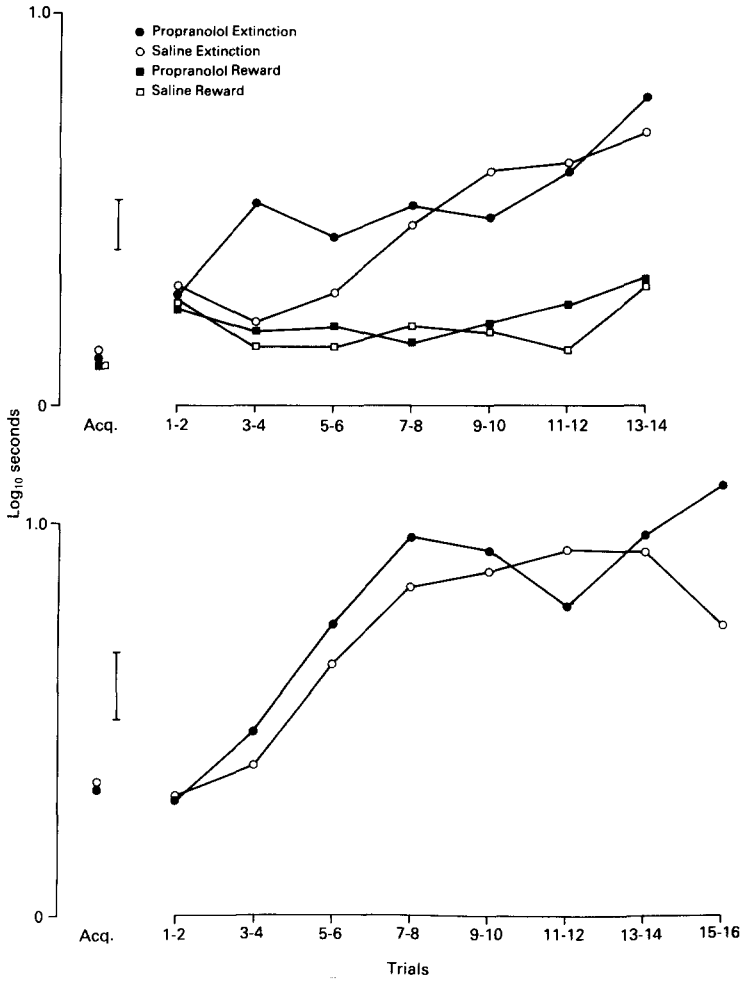

FIG. 1. Mean transformed running times in Experiments 1 (top) and 2 (bottom). Values are averaged over pairs of adjacent trials. As an index of error, bars show S.E.D.s from the analyses of variance for comparisons between drug groups on the same trial.

tween receptors and behaviour is modified by changing properties of the stress so as to increase is intensity, even when the *type* of stress is kept constant. Therefore, in the present study, the 14 extinction trials were carried out within about 30 minutes. As in our previous study, receptor binding was not clearly related to running times, but to the rate at which running times lengthened in response to nonreward. The crucial finding was that the rate of extinction correlated positively with number of both beta- and alpha$_2$-adrenoceptors. This implies that animals with the most adrenoceptors were most sensitive to the stress of nonreward.

TABLE 1

CORRELATIONS BETWEEN NEUROCHEMICAL PARAMETERS AND RATE OF INCREASE IN RUNNING TIME IN THE RUN SECTION OF THE RUNWAY AND OVER ITS TOTAL LENGTH

		Run	Total
Alpha$_2$-adrenoceptors	B_{max}	.61†	.48*
	K_d	.19	.12
Beta-adrenoceptors:	B_{max}	.45*	.58†
	K_d	.18	.39
Noradrenaline		.27	.25

*$p<0.05$; †$p<0.01$.

This result is at variance with our previous finding of a *negative* correlation between beta-adrenoceptor number and sensitivity to stress (10,12). There are two explanations for this disparity. First, it may be that the relationship depends on the intensity of stress such that there is a positive correlation between receptor density and the behavioural response to severe, but not mild, stress. This is in the direction which could be predicted from Stone's theory that beta-adrenoceptor desensitization underlies behavioural adaptation to stress. It is interesting that this theory was derived from, and continues to be supported by, studies of relatively intense forms of stress, such as repeated foot-shock and immobilization (14–16).

A second way of explaining the difference in results between our two extinction experiments arises from the different behavioural mechanisms which might operate at the different intertrial intervals. In particular, the frustration which results from nonreward may have different effects. At very short intertrial intervals, such as in the present experiment, there is evidence for an aftereffect of a nonrewarded trial which can accelerate running on

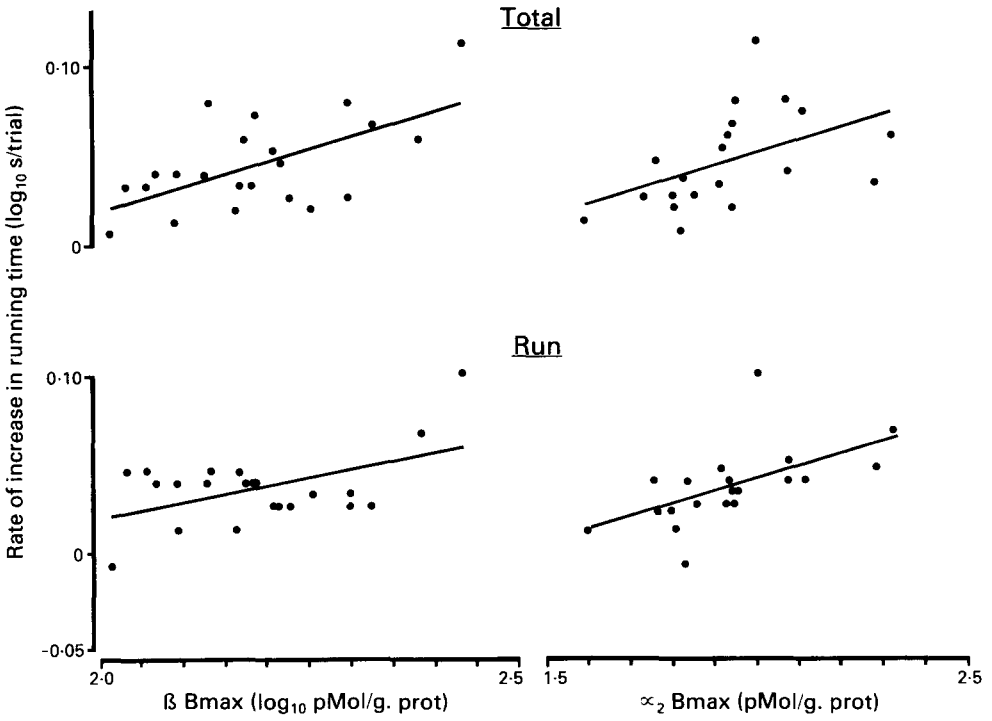

FIG. 2. Scatterplots showing relationship of $alpha_2$- and beta-adrenoceptor B_{max} with the rate of increase in running times during extinction. Linear regression lines are shown in each case.

subsequent trials [the 'frustration effect' (7,11)]. This aftereffect would not influence running speeds in experiments with 24-hour intertrial intervals, such as in our previous study; with such long intervals, the most important effect of frustration would be to condition the inhibition of running. On this basis, if receptor density is related to the intensity of a state of frustration produced by extinction trials, it might be expected to have different relationships to behaviour in the two instances. For the relationship to be opposite would, however, require the assumption that the excitatory effect of frustration at short intertrial intervals outweighs any inhibitory one. This explanation does not, therefore, seem plausible; nevertheless, one way of addressing it would be to examine the effects of modifying the intensity of the frustration, but in a different way, for example by varying the size of reward, while keeping the intertrial interval constant.

In both experiments, despite the minor differences in procedure and in the overall rates of extinction (Fig. 1), there was evidence that propranolol facilitated extinction in the early stages. This is consistent with our previous findings (9), although the subsequent effects were less stable in the present case. Notwithstanding these findings, we could find no effect of propranolol on adrenoceptor binding. Although this also supports our previous finding (10) it conflicts with others [e.g., (1,2)]. The specific conditions which permit antagonist-induced upregulation of adrenoceptors are obscure, but may depend on factors which could vary between experiments, such as the rate of tonic release of noradrenaline. In the light of our findings, however, it is unlikely that the facilitatory effect of propranolol on extinction is mediated by an upregulation of central beta-adrenoceptors (9). Nevertheless, this does not exclude the possibility that peripheral beta-adrenoceptors are involved.

Finally, noradrenaline concentration correlated only with $alpha_2$-adrenoceptor K_d; that is, it was negatively related to receptor affinity. This differs from our previous finding (12), that noradrenaline was related to $alpha_2$-adrenoceptor B_{max}. The discrepancy might be expected in view of the different time courses of the two experiments. In the present case, noradrenaline release during the experiment could have been associated with a relatively rapid desensitization of $alpha_2$-adrenoceptors, whereas in the previous experiment, a longer-term upregulation of receptor number might have occurred over the 14 days. Notwithstanding this difference from the previous results, the present finding again suggests that endogenous noradrenaline may influence $alpha_2$-, but not beta-adrenoceptors.

In conclusion, the association between greater numbers of beta-adrenoceptors and resistance to stress may be limited to the types of mild stress which we have studied previously. The relationship is reversed if more intense stress is used such that, as predicted from Stone's theory (15), *lower* beta-adrenoceptor number is associated with resistance to stress.

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