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PREVENTION AND REVERSAL OF ISOLATION-INDUCED SYSTOLIC ARTERIAL HYPERTENSION IN RATS BY TREATMENT WITH β-ADRENOCEPTOR ANTAGONISTS

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1 Rats were made hypertensive by 5 days of continuous isolation in glass metabolic cages; in the text 'hypertensive' means having a systolic blood pressure greater than 145 mmHg.

2 Daily intraperitoneal injections of either propranolol (5 mg/kg) or atenolol (5 mg/kg) reduced systolic blood pressure within 3 days and the systolic blood pressure remained low provided that the treatment was continued.

3 Treatment with metoprolol also lowered the systolic blood pressure of isolated rats but only when a larger dose (10 mg/kg) was given.

4 Systolic blood pressure returned to hypertensive levels following withdrawal of treatment after 15 days of isolation. However, following cessation of treatment after 27 days of isolation, the systolic blood pressure did not rise.

5 Rats given propranolol in the drinking water (intake equivalent to a daily dose of 5 mg/kg) before and during isolation did not develop hypertension.

6 The possibility that suppression of sympathetic function by the β-adrenoceptor antagonists was responsible for these changes is discussed.

Introduction

The literature concerning the effects of β-adrenoceptor antagonists on systemic arterial blood pressure (BP) in experimental hypertension is conflicting. In mature spontaneously hypertensive rats, propranolol has been reported to lower (Roba, Lambelin & de Schaepdryver, 1972) or to have no effect on (Forman & Mulrow, 1974, Buñag, 1977) systolic BP, and to lower (Garvey & Ram, 1975) to raise (Nakao, Kato & Takagi, 1975) or to have no effect on (Weiss, Lundgren & Folkow, 1974; Conway, Darwin Hilditch, Loveday & Reeves, 1975) mean arterial BP. Furthermore, it has been found that pretreatment with propranolol may either not affect (Frohlich & Pfeffer, 1975) or may prevent (Weiss *et al.*, 1975) the development of the spontaneous hypertension. There are reports of either a reduction (Dusting & Rand, 1974, Buñag, 1977) or no change (Farmer & Levy, 1968) in the systolic BP of deoxycorticosterone acetate (DOCA)-saline hypertensive rats, and the mean arterial BP of such hypertensive animals has been reported not to change after propranolol treatment (Conway *et al.*, 1975). However, it is generally agreed that pre-loading with propranolol does prevent the development of hypertension in young rats with DOCA implants given saline to drink (Conway *et al*, 1975; Buñag, 1977). Equally variable results come from studies on renal hypertensive rats. Using a high dose of propranolol, Leenan & Ackerman (1976) and Fernandes, Onesti, Fiorentini, Gould, Kim & Swartz (1977) reported a significant reduction in systolic BP and mean arterial BP respectively, while Niarchos, Gulati & Carretero (1977) were unable to show any effect on systolic BP in rats with established renal hypertension. However, the latter group (Niarchos *et al.*, 1977) did show that propranolol prevented the development of hypertension in 75% of the young operated animals in their experiment.

Although there is this conflict concerning the effects of β-adrenoceptor antagonists in experimental hypertension, these drugs are nonetheless effective in the treatment of many forms of human hypertension (Pritchard, 1976). It would be useful, therefore, to have an animal model of hypertension in which the antihypertensive agents are effective. Perhach, Fer-

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guson & McKinney (1975) found that propranolol lowered the systolic BP of rats made hypertensive by environmental stress, but in that study, animals were subjected to a mixture of flashing lights, cage oscillations and noise for 10 weeks before hypertension developed. Recent work in this laboratory (Gardiner & Bennett, 1977) has shown that housing rats individually in glass metabolic cages for 5 days causes a form of hypertension which has similar characteristics to stress-induced hypertension (Perhach *et al.*, 1975), but which has the obvious advantage of being simpler to produce. In the present work we have studied the effects of propranolol on the systolic BP of such isolated, hypertensive animals. In addition, the effects of atenolol and metoprolol have been investigated since there has recently been considerable clinical interest in cardioselective β_1-adrenoceptor antagonists which appear to be effective in lowering BP without having some of the undesirable side-effects of the non-selective β-antagonists (Dollery, Lewis & Myers, 1975; Åblad, Carlsson, Dahlöf & Ek, 1976; Bengtsson, 1976; De Plaen, Amery & Reybrouck, 1976; Hansson, Westerlund, Aberg & Karlberg, 1976; Petrie, Jeffers & Webster, 1977).

Methods

Male Wistar rats weighing between 200 and 250 g were used. Room temperature was between 18 and 22°C and lights were on from 07 h 30 min to 19 h 30 min. Animals were allowed free access to food (Pilsbury's diet 41B., 0.46 g% sodium) and water throughout the experiment.

Induction of hypertension by short-term isolation

Basal systolic BP was measured by the tail-cuff method (Buñag, 1977) in conscious rats daily for 1 week before isolation, and the recordings made on the last 4 days were noted. Mean pulse rate was derived from the microphone output of the BP recorder (W & W Electronics BP Recorder 8005). Rats were then transferred to individual glass metabolic cages ('Metabowl', Jencons; for details see Gardiner & Bennett, 1977) and left for 5 days in continuous isolation, after which time all animals were hypertensive (in this context 'hypertensive' means having a systolic BP greater than 145 mmHg). Subsequently, rats were housed in isolation except for a short period each day when they were grouped and handled for the measurement of BP; this is referred to as intermittent isolation. In the text the days have been numbered, day 0 being the first day of isolation. Blood pressure measurements were made between 07 h 30 min and 09 h 30 min and treatment (where appropriate) followed immediately afterwards; animals were then returned to the metabolic cages until the following morning.

The β-adrenoceptor antagonists, propranolol hydrochloride (ICI; mol. wt. 293) atenolol (ICI; mol. wt. 266) and metoprolol tartrate (Astra; mol. wt. 411) were used. All drugs were dissolved in sterile saline (0.9% w/v NaCl solution) and administered intraperitoneally; doses of propranolol and metoprolol are expressed in mg of the salt and atenolol is expressed in mg of the base.

Four separate experiments were performed and on each occasion the animals were divided into 2 groups.

Experiment 1: Effects of daily injections of saline or propranolol on systolic BP in isolated rats

Group I, ($n = 6$) Following the 5 day period of continuous isolation systolic BP was measured for 4 days whilst the animals were housed in intermittent isolation. They were then injected intraperitoneally with saline (1 ml/kg daily) for 7 days, systolic BP being measured daily just before the injection; injections were then stopped on day 15. After a further 4 days of intermittent isolation, the animals were re-housed in groups in standard cages, and systolic BP was measured for the remaining 4 days of the experiment.

Group II, ($n = 6$) Animals in this group followed the same routine to that described above, but propranolol injections were substituted for the saline. The dose of propranolol used by other workers to lower the systolic BP of hypertensive rats varies from 0.2 mg/kg subcutaneously twice daily (Dusting & Rand, 1974) to 50 mg/kg daily (Farmer & Levy, 1968). An intermediate dose of 15 mg/kg (51.3 μmol/kg) intraperitoneally was initially chosen for this pilot study. However, it was noticed that after the first drug administration the animals appeared sedated, possibly due to some central action (Leskovsky & Tardos, 1965) and therefore the dose was reduced to 5 mg/kg (17.1 μmol/kg) for the remaining 6 days of the experiment.

Experiment 2: Comparison of the antihypertensive effects of daily intraperitoneal injections of propranolol and atenolol (5 mg/kg)

Group I, ($n = 7$) Following 5 days of continuous isolation and after a 4 day control period of intermittent isolation, rats were injected with 5 mg/kg propranolol daily for 7 days to detect any influence which the initial large dose administered in the first experiment might have had on the subsequent antihypertensive action. Treatment was then stopped on day 15 for 5 days to allow recovery from the effects of propranolol. After this time systolic BP had returned to hypertensive levels. Atenolol (5 mg/kg; 18.9 μmol/kg) was then administered for a further 7 days to permit a comparison to be made between the effects of the

two β-adrenoceptor antagonists in the same animals. When the treatment was stopped (day 27), the animals remained in isolation for a further 4 days before being grouped for the remaining 2 days of the experiment. Systolic BP was measured daily throughout.

Group II, ($n = 5$) The second group of rats underwent the same treatment as described above except that atenolol (5 mg/kg daily for 7 days) was given as the first β-antagonist and propranolol (5 mg/kg daily for 7 days) as the second drug.

Experiment 3: Comparison of the antihypertensive effects of daily intraperitoneal injections of metoprolol and propranolol (5 mg/kg)

Group I, ($n = 7$) After 5 days of continuous isolation and 4 control days of intermittent isolation, rats were injected with metoprolol (5 mg/kg; 12.15 μmol/kg) daily for 7 days to compare the effect of this cardioselective β-adrenoceptor antagonist with that of atenolol (Experiment 2, Group II). Treatment was then stopped on day 15 for 5 days before the animals were injected with propranolol (5 mg/kg daily) for 7 days; this was done since the antihypertensive action of propranolol had already been established and thus it permitted a direct comparison to be made between the two drugs in the same animals. Rats remained in intermittent isolation for a further 4 days after the last propranolol injection (day 27) before being grouped for the last 2 days of the experiment.

Group II, ($n = 5$) The remaining animals in this experiment were treated initially with propranolol (5 mg/kg daily for 7 days) and subsequently with metoprolol (5 mg/kg daily for 7 days). The metoprolol injections were continued for a further 4 days with twice the dose (10 mg/kg; 24.3 μmol/kg). Rats were then grouped for the remaining 2 days of the experiment.

Experiment 4: Effects of metroprolol (10 mg kg^{-1} day^{-1}) on systolic BP in isolated rats

The results of Experiment 3 showed that metoprolol (5 mg/kg) did not significantly affect the systolic BP of isolated rats (see Results). The purpose of this last experiment was to study the effects of a larger dose of the drug.

Group I, ($n = 6$) Following 5 days of continuous isolation and after 4 control days of intermittent isolation, rats were injected with metoprolol (10 mg/kg; 24.3 μmol/kg) daily for 7 days. Treatment was then stopped on day 15 and systolic BP was measured for a further 3 days of intermittent isolation.

Group II, ($n = 4$) The remaining animals in this experiment were treated as described above but saline injections were given instead of metoprolol.

Effects of pretreatment with propranolol before isolation

Systolic BP was measured daily for 4 days whilst the animals ($n = 12$) were housed in groups of 4 in standard cages, and daily water intakes of the groups were recorded. A solution of propranolol (10 mg dissolved in 250 ml water) was then substituted for the drinking water and presented to the groups of rats for a further 5 days; systolic BP and propranolol intakes were measured daily. Animals were then transferred to individual metabolic cages and left for 5 days in continuous isolation, still with a propranolol solution substituted for drinking water. The concentration of propranolol in the water was adjusted according to the mean water intakes of rats housed under similar conditions (unpublished observations) such that each rat received a dose of approximately 5 mg kg^{-1} day^{-1}; the actual intake was noted. Systolic BP was then measured for 3 days during which time the animals were housed in intermittent isolation with propranolol in the drinking water.

Control animals ($n = 6$) received tap water to drink throughout the experiment.

Data analysis

Values are expressed as the mean ±1 standard error of the mean (s.e. mean); n is the number of animals. Differences were tested for statistical significance by Student's paired t test.

There was no significant difference between the values of systolic BP recorded on the last 4 days before isolation in any of the groups of animals; measurements made on the last day, therefore, were taken as the baseline values. Likewise, there was no significant difference between the recordings made during the 4 control days of intermittent isolation before treatment; thus, measurements made on the fourth day were used as the control values. In the text, the term 'baseline' refers to measurements made before isolation whereas 'control' values are those obtained during intermittent isolation.

Results

Induction of hypertension by short-term isolation

Five days of continuous isolation in metabolic cages caused a significant systolic arterial hypertension in all groups of animals (Figures 1 to 3; Table 1). There was also an increase in heart rate but the change was not always significant (Table 1).

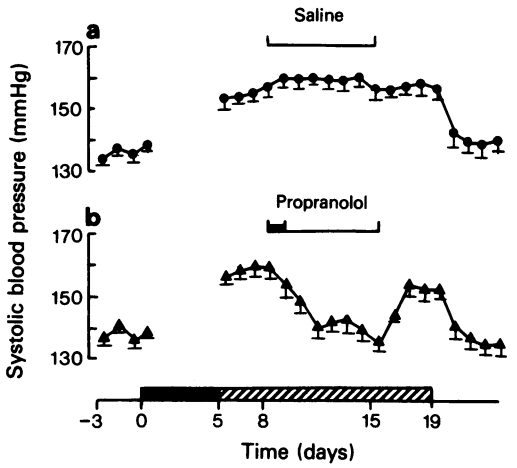

Figure 1 The effects of daily injections of saline (a; ●, $n = 6$) and propranolol (b; ▲, $n = 6$) on systolic blood pressure in rats. Lines above the graphs correspond to periods of treatment (propranolol, 5 mg/kg i.p.; saline, 1 ml/kg i.p.). The day when the dose of propranolol was 15 mg/kg is represented by a thick line. Five days of continuous isolation (■) caused a significant increase in systolic blood pressure in both groups of animals and subsequent intermittent isolation (■) did not alter the blood pressure of saline-treated rats. Daily injections of propranolol reduced the blood pressure to normotensive levels within 3 days and withdrawal of treatment after 7 days caused blood pressure to return to hypertensive levels. Grouping after 19 days of isolation lowered blood pressure in both groups of animals.

Experiment 1: Effects of daily injections of saline or propranolol on systolic BP in isolated rats

Group I In saline-treated animals the systolic BP remained elevated throughout the 14 days of intermittent isolation (following 5 days of continuous isolation), but fell within 24 h after the animals were grouped, to a level not significantly different from the baseline value (Figure 1a). The increase in heart rate seen after 5 days of isolation was significant (for values see Table 1) but this change was not maintained; after a further 4 days of intermittent isolation, the mean heart rate was not significantly different from the baseline value (this phenomenon has been discussed elsewhere, Gardiner & Bennett, 1977). Due to these changes in heart rate, any possible effects of β-antagonists on this variable could not reliably be detected, so heart rate data were not systematically analysed.

Group II Propranolol (15 mg/kg on day 1 followed by 5 mg kg^{-1} day^{-1} for 6 days) caused a gradual reduction in systolic BP (Figure 1b). The systolic BP was significantly less than the control level on the day after the second injection ($0.01 > P > 0.001$; $n = 6$) and it remained low throughout the rest of the treatment period. The last dose of propranolol was given on day 14 of the experiment and systolic BP was still low 48 h later, but after 72 h it had risen to a level not significantly different from control (Figure 1b). When the animals were grouped after a further 48 h of intermittent isolation, systolic BP fell to baseline levels within 24 h (Figure 1b).

Table 1 Mean (±s.e. mean) values for systolic blood pressure and heart rate in rats before (baseline) and after (control) 5 days of continuous isolation

	Systolic blood pressure (mmHg)		*Heart Rate (beats/min)*	
	Baseline	*Control*	*Baseline*	*Control*
Experiment 1				
Group I	138 ± 2.7	154 ± 4.4****	353 ± 14.9	387 ± 10.4*
Group II	138 ± 1.19	156 ± 1.68****	351 ± 8.95	408 ± 9.53**
Experiment 2				
Group I	137 ± 2.92	151 ± 0.93****	354 ± 7.5	388 ± 28.5
Group II	140 ± 1.22	166 ± 3.40****	359 ± 15	397 ± 21*
Experiment 3				
Group I	137 ± 4.0	159 ± 4.2****	385 ± 14.1	375 ± 9.3
Group II	139 ± 3.7	159 ± 3.8****	355 ± 16.2	419 ± 12.6**
Experiment 4				
Group I	135 ± 3.2	158 ± 4.8****	321 ± 6.5	398 ± 12.2**
Group II	135 ± 2.2	153 ± 3.1****	429 ± 15.2	408 ± 22.1*

* $0.05 > P > 0.02$; ** $0.02 > P > 0.01$; **** $P < 0.001$; by Student's paired t test.

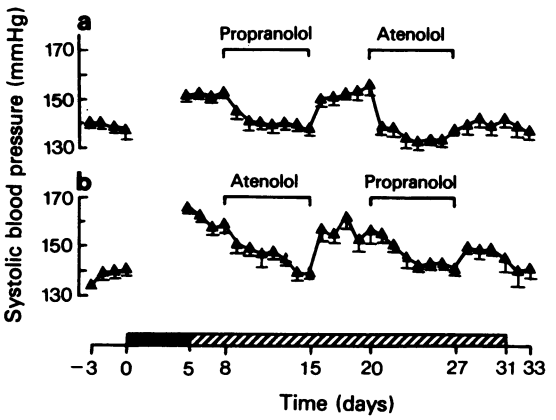

Figure 2 Systolic blood pressure in rats made hypertensive by 5 days of continuous isolation (■) and subsequently given daily intraperitoneal injections of β-adrenoceptor antagonists (represented by lines above the graphs) whilst being housed in intermittent isolation (■). There was no detectable difference between the blood pressure changes in rats given firstly propranolol (5 mg kg^{-1} day^{-1}) and secondly atenolol (5 mg kg^{-1} day^{-1}; $n = 7$) shown in (a) or *vice versa* ($n = 5$) shown in (b).

Experiment 2: Comparison of the antihypertensive effects of daily intraperitoneal injections of propranolol and atenolol (5 mg/kg)

Group I Daily injections of propranolol (5 mg/kg) significantly lowered the systolic BP within 3 days ($0.05 > P > 0.02$; $n = 7$) and kept the BP at a low level for the remaining 5 days of treatment. This result is directly comparable to that described above (Experiment 1, Group II) and indicates that the initial high dose of propranolol was not required for the ultimate antihypertensive effect. The last dose of propranolol was given on day 14 and 24 h later the systolic BP had risen to a level not significantly different from the control value and it remained high during the following 4 days of intermittent isolation (Figure 2a). Atenolol (daily dose of 5 mg/kg) abolished the hypertension within 24 h ($0.02 > P > 0.01$; $n = 7$) and BP remained low whilst treatment continued. Cessation of the atenolol injections after 27 days of isolation and also subsequent grouping after a further 4 days did not significantly affect the BP (Figure 2a). This finding differs from the results in Experiment 1 (Group II) which showed that following withdrawal of treatment on day 14, systolic BP rose and with grouping on day 19 it fell again.

Group II Changes similar to those described above were also seen in rats given firstly atenolol (5 mg/kg daily for 7 days) and secondly propranolol (5 mg/kg daily for 7 days; Figure 2b). The reduction in systolic BP caused by atenolol was significant on the day after the third injection ($0.05 > P > 0.02$; $n = 5$) and was sustained for the following 4 days of treatment. The last dose of atenolol was given on day 14 and systolic BP rose within 24 h and remained high for the following 4 days of intermittent isolation. After the third and subsequent injections of propranolol (5 mg/kg), systolic BP was significantly less than control ($0.01 > P > 0.001$; $n = 5$). As above, when treatment was finally stopped (day 27) and also when the animals were grouped (day 31), systolic BP did not change significantly (Figure 2b).

Collectively, the results of Experiment 2 showed that atenolol and propranolol were equally effective in lowering the systolic BP of isolated rats.

Experiment 3: Comparison of the antihypertensive effects of daily intraperitoneal injections of propranolol and metoprolol (5 mg/kg)

Group I Daily intraperitoneal injections of metoprolol (5 mg/kg) for 7 days did not significantly alter the systolic BP of isolated, hypertensive rats ($P > 0.5$) although there was a slight reduction in systolic BP during the first 3 days of treatment (Figure 3a). However, in the same animals propranolol (5 mg/kg) given daily for 5 days after the last dose of metoprolol did lower systolic BP (Figure 3a); the change was significant on all days after the third and subsequent injections ($P < 0.001$; $n = 7$). Treatment with propranolol was stopped on day 27 and systolic BP rose but it did not reach a significantly higher level before the animals were grouped (Figure 3a).

Group II Rats in this part of the experiment received the same 2 drugs as above but in reverse order. Initially they showed a reduction in systolic BP in response to propranolol (5 mg/kg daily) which was significant after the third day of treatment onwards ($0.01 > P > 0.001$; $n = 5$, Figure 3b). The last propranolol injection was given on day 14 and systolic BP rose to a level not significantly different from the control value 48 h later, and remained high for a further 2 days of intermittent isolation. As before, daily injections of metoprolol (5 mg/kg, i.p.) for 7 days did not significantly affect the hypertension although, again, there was a slight reduction in systolic BP during the first 3 days of treatment. Furthermore, a dose of 10 mg/kg daily for 4 days did not alter systolic BP. When the drug administration was finally stopped and the animals grouped (day 31), systolic BP did not fall (Figure 3b).

Thus the results of Experiment 3 show that daily intraperitoneal injections of 5 mg/kg of metoprolol for 7 days do not have the same antihypertensive properties in isolated rats as injections of atenolol or propranolol given in the same dose and by the same route.

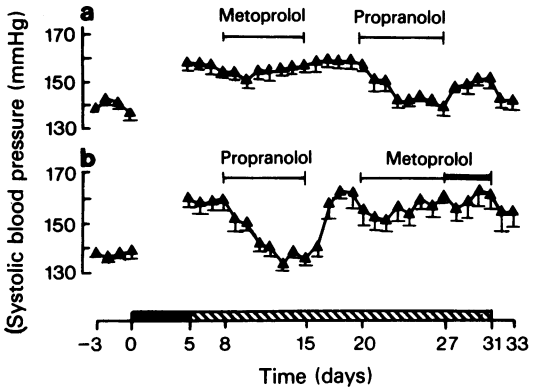

Figure 3 Systolic blood pressure in rats made hypertensive by 5 days of continuous isolation (■) and subsequently given daily intraperitoneal injections of β-adrenoceptor antagonists (represented by lines above the graphs) whilst being housed in intermittent isolation (■). Metoprolol (5 mg kg^{-1} day^{-1} for 7 days) did not alter the blood pressure of hypertensive rats (a, $n = 7$) whereas propranolol treatment (5 mg kg^{-1} day^{-1}) in the same animals lowered systolic blood pressure. Similarly, initial treatment with propranolol (5 mg kg^{-1} day^{-1}, b, $n = 5$) lowered systolic blood pressure but subsequent treatment with metoprolol (5 mg/kg) did not alter blood pressure; further treatment with 10 mg/kg metoprolol for 4 days (|=|) was still ineffective in lowering blood pressure.

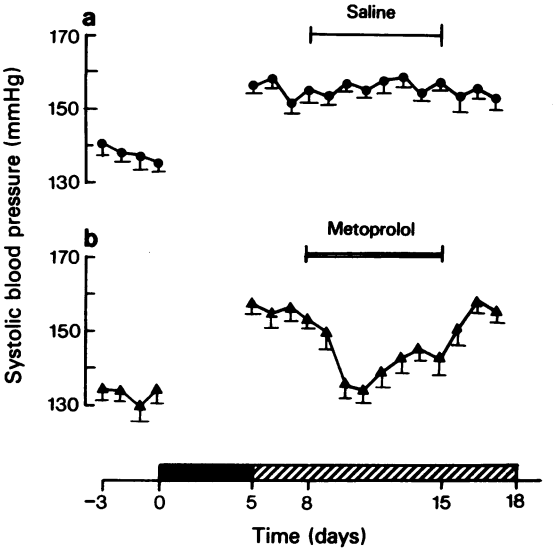

Figure 4 The effects of daily injections of saline (●, 1 ml kg^{-1} day^{-1}, $n = 4$) and metoprolol (▲, 10 mg kg^{-1} day^{-1}, $n = 6$) on systolic blood pressure in rats made hypertensive by 5 days of continuous isolation (■). Systolic blood pressure fell within 2 days after the onset of the metoprolol treatment and remained low while treatment was continued.

Experiment 4: The effect of metoprolol (10 mg kg^{-1} day^{-1}) on systolic BP in isolated rats

Group I Daily injections of metoprolol (10 mg/kg, i.p.) starting on day 8 significantly lowered the systolic BP within 2 days ($P < 0.001$; $n = 6$, Figure 4b). Blood pressure remained lower than the control level while the treatment continued but the difference was not always significant (days 5 and 6). Following cessation of treatment on day 15, systolic BP returned to hypertensive levels within 24 h (Figure 4b).

Group II The hypertension induced by 5 days of continuous isolation persisted throughout the 7 days of saline injections (Figure 4a).

The results of Experiment 4 show that the higher dose of metoprolol (10 mg/kg), although ineffective in lowering systolic BP in the later stages of hypertension (Experiment 3, Group II) did nevertheless reduce systolic BP after a shorter period of isolation.

Effects of pre-treatment with propranolol before isolation

Substitution of a propranolol solution (0.04 mg/ml) for drinking water did not affect the systolic BP or heart rate of normotensive rats housed in groups (Figure 5). After 5 days of continuous isolation in metabolic cages, still with propranolol in the drinking water, none of the animals was hypertensive (Figure 5). In contrast, the systolic BP of control animals given tap water to drink was significantly elevated after 5 days of continuous isolation (before isolation = 135 ± 2.06 mmHg; after 5 days of isolation = 154 ± 3.16 mmHg: $P < 0.001$; $n = 6$). Similarly, whereas the heart rate of control animals was significantly higher after 5 days of isolation, (before isolation = 341 ± 6.2 beats/min; after isolation = 401 ± 12.9 beats/min; $0.02 > P > 0.01$, $n = 6$), rats pretreated with propranolol showed no significant change in heart rate (before isolation = 338 ± 11.5 beats/min; after isolation = 348 ± 12.3 beats/min; $n = 12$) (Figure 5).

Discussion

Results described here confirm earlier observations in this laboratory (Gardiner & Bennett, 1977) that short-term isolation of rats in metabolic cages causes systolic arterial hypertension which is sustained (provided that isolation is continued), but which can be reversed by re-housing the animals in groups within a 2 week period of isolation. The present studies also show that hypertension can be reversed by treatment

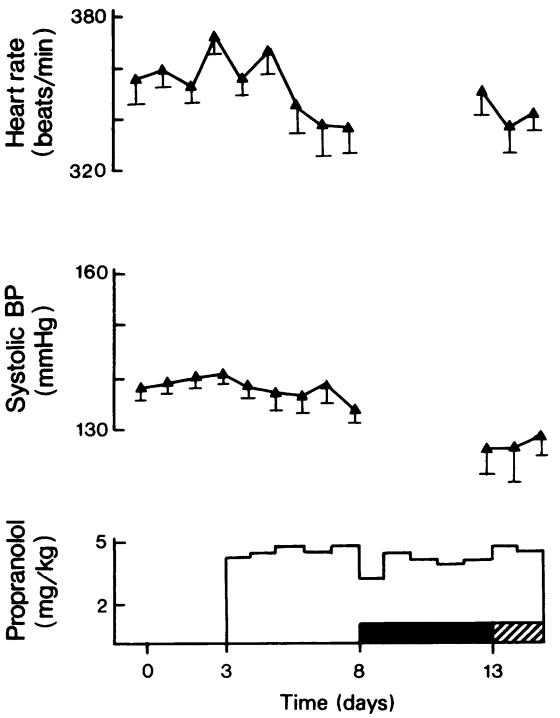

Figure 5 Systolic blood pressure and heart rate of rats ($n = 12$) given propranolol before and during 5 days of continuous isolation. A propranolol solution of known concentration was substituted for the drinking water and the intake (and hence dose) was measured daily. Systolic blood pressure and heart rate were unaffected whilst the animals were housed in groups. Also, after 5 days of continuous isolation (■) and during a further 3 days of intermittent isolation (▨), systolic blood pressure and heart rate were unchanged.

with propranolol, atenolol or metoprolol and can be prevented by pretreatment with propranolol.

The conflicting results of other workers (see Introduction) on the effects of β-adrenoceptor antagonists in experimental hypertension may be due partly to the differing aetiologies of hypertension in the various experimental models, and partly to inconsistencies in a number of variables which should be taken into consideration. Standardization of both the dose and the route of drug administration and also the time between treatment and BP measurement is essential if meaningful comparisons are to be made.

In the present study, when such precautions were taken, it was found that daily injections of 5 mg/kg of either propranolol or atenolol were equally effective in lowering the systolic BP of isolated, hypertensive rats, whereas the same dose of metoprolol was without significant effect. Propranolol, atenolol and metoprolol are stated to be equipotent (per mg of drug) in their antihypertensive action (Lewis, 1976). However, further examination of the original data cited in the review by Lewis (1976) shows that the dose of metoprolol (mg) used previously (Johnsson, 1975) was larger than that of propranolol. Since the molecular weight of metoprolol tartrate (411) is greater than that of propranolol hydrochloride (293) then the higher dose of metoprolol used by most workers (Johnsson, 1975; Åblad, *et al.*, 1976; Johnsson & Regårdh, 1976; Levy, 1976; Raine & Chubb, 1977) is roughly equimolar with the dose of propranolol used. In the present work it was found that daily intraperitoneal injections of 10 mg/kg metoprolol did lower the systolic BP of isolated rats when given in the early stages of the hypertension. The same dose given after a longer period of isolation did not alter the systolic BP but at that time, grouping the animals did not affect the systolic BP either and so it is possible that the hypertension had reached an irreversible stage.

There was a delay in the onset of the antihypertensive action of the β-adrenoceptor blocking agents such that systolic BP was generally not significantly lowered until after the second or third injection; this phenomenon has been well documented for both non-selective and cardioselective β-blockers in hypertensive rats (Dusting & Rand, 1974; Garvey & Ram, 1975; Perhach *et al.*, 1975; Ljung, Åblad, Drews, Fellenius, Kjellstedt & Wallborg, 1976; Buñag, 1977) and as Åblad *et al.* (1976) have pointed out, this argues against the possibility that the antihypertensive action was the direct result of cardiac β-adrenoceptor blockade. Since the mechanism of the antihypertensive action of β-receptor antagonists is still unknown (Hollifield, Sherman, Zwagg & Shand, 1976; Lewis, 1976; Amer, 1977) it is not possible to say how they lowered the systolic BP of our isolated rats. It is unlikely that they acted through suppression of renin release since plasma renin levels are not elevated in isolated hypertensive animals (Gardiner & Husain, unpublished) and it is unlikely that the antihypertensive effect was centrally mediated since atenolol is reported not to cross the blood-brain barrier (Bühler, Burkart, Lütold, Küng, Marbet & Pfisterer, 1975) whereas it was as effective as propranolol in lowering BP. The hypertension induced by short-term isolation is abolished by chemical sympathectomy with 6-hydroxydopamine (Bennett & Gardiner, 1978a), and is characterized by hyperactivity of the adrenal cortex (Bennett & Gardiner, 1978b). Elevated corticosteroid levels in isolated rats are associated with increased noradrenergic responsiveness of cardiovascular tissues, perhaps due to changes in tissue electrolyte distribution (Bennett & Gardiner, 1978b). It is likely, therefore, that the hypertension seen during the early stages of isolation is due to increased sympathetic nerve activity acting on sensitized cardiovascular tis-

sues. This would explain why the hypertension is reversed by 6-hydroxydopamine-induced interference with the sympathetic nervous system even though corticosteroid levels and tissue sensitivities might remain high. Åblad *et al.* (1976) suggested that β-adrenoceptor antagonists acted by preventing neuronal release of noradrenaline, thereby lowering peripheral vascular tone, and Raine and his colleagues (Raine & Chubb, 1977; Raine & Pickering, 1977) have recently shown a réduction in the activity of both tyrosine hydroxylase and dopamine-β-hydroxylase in sympathetic ganglia after chronic treatment with β-blockers. We would suggest, therefore, that reduced sympathetic nerve function may have been responsible for the antihypertensive effects of β-blockers shown in the present study.

Presumably, the antihypertensive effect of grouping the animals in the early stages of the hypertension is also due to a reduction in sympathetic drive. However, after prolonged periods of isolation it appears that the underlying basis for the hypertension changes, since grouping animals after 30 days of isolation does not affect systolic BP. Rosencrans, Watzman & Buckley (1966) found that with chronic environmental stress, hyperactivity of the adrenal cortex was still evident at the end of the 16 week period of study whereas sympathetic nerve hyperactivity, assessed by plasma and urinary catecholamine levels, declined after the fourth week of stress. Thus it is possible that in isolated rats the role of the sympathetic nervous system in maintaining the hypertension wanes at around 30 days and morphological changes in the peripheral vasculature due to the high pressure load (Folkow, Hallbäck, Lundgren, Sivertsson & Weiss, 1973) might become more important in maintaining the high BP. If this were so then it would account for the present finding that at these later stages a high systolic BP was not reduced by metoprolol or by grouping, whereas a low pressure, maintained by β-adrenoceptor antagonists, did not rise on cessation of treatment.

From the present findings, it is not valid to assume that the mechanism by which the β-adrenoceptor antagonists effect a reduction in BP is the same in clinical hypertension (which usually involves an elevated diastolic as well as systolic pressure) and in isolation-induced hypertension (in which only systolic B.P. was measured). However, it is noteworthy that the effective doses used in the present study (propranolol 5 mg/kg; atenolol 5 mg/kg, metoprolol 10 mg/kg) are only marginally greater than the therapeutic doses (propranolol 1 to 4.5 mg/kg; atenolol 1.5 to 2 mg/kg; metoprolol 1.5 to 6 mg/kg), a finding which adds weight to the possible clinical relevance of the present work.

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