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# B-HT 958 lowers blood pressure and heart rate in the rat through stimulation of dopamine receptors

Morris J. Brown & Deborah Harland

Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 0HS

**1** To investigate whether the hypotensive and bradycardiac effects of B-HT 958 (2-amino-6-(p-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo-(5,4-d) azepine) are due to the stimulation of peripheral prejunctional $\alpha_2$-adrenoceptors, the selective $\alpha_2$-adrenoceptor antagonist idazoxan was given either intravenously (i.v.) or intracerebroventricularly (i.c.v.) to anaesthetized rats before the administration of i.v. B-HT 958. Plasma noradrenaline was used as an approximate index of peripheral sympathetic nervous activity.

**2** B-HT 958 350 $\mu g\,kg^{-1}$ i.v. caused significant falls in blood pressure and heart rate which were maximal 5 min after dosing ($-$ 29.25 $\pm$ 3.2 mmHg and $-$ 52 $\pm$ 6.8 beats $min^{-1}$ respectively, mean of all control animals). The hypotension and bradycardia were accompanied by significant falls in plasma noradrenaline concentration of 30–40%.

**3** Idazoxan 300 $\mu g\,kg^{-1}$ i.v. caused a marked, but transient tachycardia and a large sustained rise in plasma noradrenaline concentration. Idazoxan 300 $\mu g\,kg^{-1}$ and 1000 $\mu g\,kg^{-1}$ i.v. did not prevent B-HT 958-induced falls in mean arterial pressure, heart rate and plasma noradrenaline concentration. Responses to B-HT 958 were unaffected by idazoxan 20 $\mu g$ i.c.v.

**4** B-HT 958-induced falls in mean arterial pressure, heart rate and plasma noradrenaline concentration were significantly attenuated by i.v. administration of the dopamine receptor antagonist, sulpiride 300 $\mu g\,kg^{-1}$. Sulpiride 10 $\mu g$ and 50 $\mu g$ i.c.v. caused inhibition of B-HT 958 hypotension and bradycardia similar to that of intravenous sulpiride. After i.c.v. sulpiride, B-HT 958 did not cause a significant fall in plasma noradrenaline concentration.

**5** A combination of idazoxan 1000 $\mu g\,kg^{-1}$ i.v. and sulpiride 300 $\mu g\,kg^{-1}$ i.v. did not cause further significant inhibition of B-HT 958 hypotension and bradycardia compared with sulpiride 300 $\mu g\,kg^{-1}$ i.v. alone. This combination however had a significantly greater effect in inhibiting B-HT 958 hypotension than had idazoxan 1000 $\mu g\,kg^{-1}$ alone, and almost completely blocked the B-HT 958-induced fall in plasma noradrenaline concentration.

**6** These results suggest that in the anaesthetized rat the cardiovascular effects of B-HT 958 are due to stimulation of dopamine receptors, probably located within the central nervous system, and not to stimulation of peripheral prejunctional $\alpha_2$-adrenoceptors.

## Introduction

In a recent report, B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-(54-d) azepine) was described as a partial agonist at $\alpha_2$-adrenoceptors (Pichler *et al.*, 1982). In the pithed rat B-HT 958 showed agonist activity at prejunctional $\alpha_2$-adrenoceptors, inhibiting tachycardia evoked by low frequency stimulation of the spinal sympathetic outflow. In the same dose-range at vascular postjunctional sites B-HT 958 acted mainly as an $\alpha_2$-adrenoceptor agonist. The apparent pre-postjunctional $\alpha_2$-adrenoceptor agonist activity ratio of B-HT 958 is therefore very high (Pichler *et al.*, 1982).

BHT-958 is chemically related to the 'clonidine-like' drugs B-HT 920 and B-HT 933 (Kobinger & Pichler, 1977; Hammer *et al.*, 1980). and has been shown to lower blood pressure and heart rate in anaesthetized cats and rats by a mechanism interpreted as involving $\alpha$-adrenoceptor stimulation (Kobinger & Pichler 1984). The contribution of central nervous system sites to the hypotensive effect of B-HT 958 was believed to be small and appeared to be located in areas rostral to the pons and medulla. In addition, the ability of B-HT 958 to lower blood pressure and heart rate in decerebrate cats without a concomitant decrease in the

discharge rate of the sympathetic splanchnic nerve (Kobinger & Pichler, 1984), indicated that B-HT 958 was unlike clonidine in its hypotensive action and that peripheral mechanisms of the drug may be responsible for these cardiovascular effects rather than changes in sympathetic nerve activity within the brainstem. Adrenergic neurone and ganglion blocking actions of B-HT 958 were excluded as possible causes of the hypotension and bradycardia (Kobinger & Pichler, 1984). It has been suggested that B-HT 958 may reduce release of noradrenaline prejunctionally without causing postjunctional vasoconstriction (Kobinger & Pichler, 1984). If this is correct, it may indicate a role for prejunctional $\alpha_2$-adrenoceptors in the pharmacological control of peripheral vascular tone and B-HT 958 may therefore represent a new class of antihypertensive drug.

In the present study we have used the selective $\alpha_2$-adrenoceptor antagonist, idazoxan (Doxey *et al.*, 1983) to investigate whether the hypotensive effect of B-HT 958 is due to an agonist action at peripheral prejunctional $\alpha_2$-adrenoceptors. Plasma noradrenaline was used as an approximate index of peripheral sympathetic nervous activity. It was anticipated that intracerebroventricular (i.c.v.) administration of idazoxan would have little effect on B-HT 958-induced changes in blood pressure, heart rate and plasma noradrenaline if they were mediated by a peripheral action, whereas intravenous (i.v.) idazoxan would antagonize these effects.

It is also possible that B-HT 958 may possess dopamine receptor agonist properties, as the chemically related compound B-HT 920 was recently found to be a potent agonist at prejunctional dopamine receptors in the central and peripheral nervous systems (Anden *et al.*, 1982; 1983; Willfert *et al.*, 1984). We have therefore studied the effect of central and peripheral administration of the dopamine receptor antagonist sulpiride on the cardiovascular responses to B-HT 958.

## Methods

### *Animals and general procedures*

Male normotensive Wistar rats (250–350 g, Charles River, U.K.) were anaesthetized with Inactin (sodium salt of 5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid) 100 mg kg$^{-1}$ i.p., an anaesthetic without any hypotensive action (Munoz-Ramirez *et al.*, 1978). The trachea was cannulated and polyethylene catheters were placed in the right external jugular vein and the ipsilateral common carotid artery. The arterial catheter was advanced so that the tip lay in the aortic arch and arterial blood pressure was recorded via a Statham P23 ID or Bell & Howell type 4-422 pressure transducer attached to a Grass model 79D polygraph. Pulsatile blood pressure was recorded continuously. Heart rate was calculated directly from the blood pressure recording every 5 min by increasing the chart speed and counting the number of beats occurring in 10 s. Body temperature was maintained by placing the animals on a thermostatically controlled heating pad ('Thermega', Remploy UK) set at 38°C.

### *Administration of drugs*

In preliminary experiments cumulative dose-response curves to i.v. B-HT 958 were constructed according to the following schedule: at 0 min, isotonic saline (1 ml kg$^{-1}$) was administered. Doses of B-HT 958 of 62.5, 62.5, 125, 250, 500 and 1000 $\mu$g kg$^{-1}$ were then administered successively at 10, 20, 30, 40, 50 and 60 min respectively. Mean blood pressure and heart rate responses were determined 10 min after injection of each dose.

From the dose-response curves, a dose of B-HT 958 producing a fall in mean arterial pressure of approximately 30 mmHg, i.e. 350 $\mu$g kg$^{-1}$ i.v., was chosen for use in the studies with antagonists. This dose of B-HT 958 caused similar reductions in blood pressure, heart rate and plasma noradrenaline concentration as clonidine 5 $\mu$g kg$^{-1}$ i.v. (Brown & Harland, 1984). Idazoxan, (RS)-sulpiride, a combination of idazoxan and sulpiride or saline were administered i.v. in a total volume of 1 ml kg$^{-1}$ as a 5 min infusion. Intracerebroventricular administration of idazoxan 20 $\mu$g and sulpiride 10 $\mu$g and 50 $\mu$g was made via a 30G stainless steel needle attached to a Hamilton microsyringe. Animals were placed in a David Kopf stereotaxic frame and a bore hole drilled in the skull 1.7 mm lateral and 1 mm posterior to bregma. Injections were made in a total volume of 10 $\mu$l given over 5 min at a depth of 4 mm from the dura. Control animals received 10 $\mu$l of saline. Verification of i.c.v. injection sites was made at the end of the experiment by injecting 10 $\mu$l of Evans Blue dye and examination of the brain for staining of the ventricular spaces. B-HT 958 350 $\mu$g kg$^{-1}$ i.v. or saline were administered 5 min after the end of the i.v. or i.c.v. infusion of the antagonists or saline.

Both the i.v. and i.c.v. doses of idazoxan used have been shown in previous experiments to be selective for $\alpha_2$-adrenoceptors, and also to inhibit effectively the cardiovascular effects of clonidine in anaesthetized rats (Berridge *et al.*, 1982; Doxey *et al.*, 1983; 1984; Brown & Harland, 1984). Sulpiride was given at doses that antagonize both the cardiovascular actions of, and behavioural responses to $DA_2$-dopamine receptor agonists (Sved & Fernstrom, 1980; Barrett & Lockhandwala, 1981; Cavero, 1981; Cavero *et al.*, 1981a, 1982a,b, 1984; Nishibe *et al.*, 1982).

Previous experiments (Berridge *et al.*, 1982; Brown

& Harland, 1984) have shown that low i.c.v. doses of idazoxan have no peripheral actions. In order to confirm a similar lack of peripheral effects of sulpiride, we investigated whether i.c.v. sulpiride antagonized the depressor responses to i.v. dopamine in rats pretreated with a combination of propranolol 1 mg kg$^{-1}$ i.v. and phentolamine 5 mg kg$^{-1}$ i.v. infused over a total time of 6 min. This combination was effective in causing greater than 90% inhibition of the pressor response to noradrenaline 0.5 μg kg$^{-1}$ i.v. for approximately 30 min. The depressor responses to dopamine 25, 50 and 100 μg kg$^{-1}$ i.v. were then determined in the presence of i.v. or i.c.v. sulpiride.

*Determination of plasma levels of noradrenaline*

In the dose-response experiments with B-HT 958, blood samples (0.35 ml) were withdrawn from the carotid arterial cannula 5 min after each dose of B-HT 958. In the antagonist experiments blood samples were taken 5 min before and immediately after idazoxan, sulpiride or saline, and 5, 10, 30 and 60 min after B-HT 958 350 μg kg$^{-1}$ i.v. Blood was replaced with an equal volume of saline. The blood samples were centrifuged at 4°C for 15 min, the plasma immediately separated and stored at − 80°C until assayed. Plasma concentration of noradrenaline was determined in duplicate for each sample by a double isotope enzymatic assay (Brown & Jenner, 1981).

*Drugs*

Inactin (5-ethyl-5-(1-methylpropyl)-2-thiobarbitone sodium) was a gift from Dr Pittman, BYK Gulden, Konstanz, W.Germany. Idazoxan (2-[2-(1,4-benzodioxanyl)]-2-imidazoline hydrochloride) was a gift from Dr J.C. Doxey, Reckitt & Colman plc, Hull, B-HT 958 (2-amino-6-(*p*-chlorobenzyl)-4H-5,6,7,8-tetrahydrothiazolo-(5,4-d) azepine dihydrochloride) from Dr Karl Thomae GmbH, Biberach an der Riss, W.Germany, and (RS)-sulpiride from Delagrange, Paris, France. Propranolol hydrochloride was obtained from ICI plc, Macclesfield, Cheshire and phentolamine mesylate (Rogitine injection) from Ciba, Horsham. Inactin was dissolved in sterile water for injection (Kirby-Warwick). Idazoxan, B-HT 958 and propranolol were dissolved in 0.9% w/v saline. Sulpiride was dissolved in 200–400 μl of 0.1 M HCl, the pH adjusted to 7.4 with NaOH and made up to volume with distilled water. Noradrenaline acid tartrate (Levophed Special Solution, Winthrop Laboratories) and dopamine hydrochloride (Intropin, American Hospital Supply UK Ltd.) were diluted in 0.9% w/v saline containing ascorbic acid 1 mg ml$^{-1}$. All doses in the text refer to the respective salts.

*Statistical analysis*

Results are expressed as mean ± s.e.mean where *n* refers to the number of observations. The effect of B-HT 958 on plasma noradrenaline concentration within experimental groups was tested for significance using oneway analysis of variance for repeated measurements (ONEWAY) (Wallenstein *et al.*, 1980). This test was also used to assess the effects of the antagonists alone on plasma noradrenaline concentration. Absolute values of plasma noradrenaline (ng ml$^{-1}$) were used in the analysis. Significance of differences in the size of the fall in plasma noradrenaline produced by B-HT 958 between control groups and antagonist-treated groups were determined using two way analysis of variance for repeated measurements (TWOWAY). Repeated measurements analysis of variance is used when several measurements are made pre- and post-intervention in the same experimental group. The oneway test indicates the degree of variation between the time points within each group. The twoway test is used to assess the effects of a second variable, e.g. antagonist treatment, on the time trends.

A combination of oneway analysis of variance and an unpaired two-tailed Student's *t* test (Bonferroni methods for multiple comparisons as described by Wallenstein *et al.*, 1980) was used to test for significant inhibition of B-HT 958 hyoptension and bradycardia by the various antagonist treatments. Changes in mean arterial pressure and heart rate from baseline were used in this analysis. The Bonferroni test makes use of information concerning variability within experimental groups. The analysis of variance gives a single test statistic for differences between all groups tested. If this test indicates that some differences exist, modified *t* tests are then performed on a pre-planned number of comparisons to identify the source of the differences. The Bonferroni method was also used to test for significant effects of the antagonists alone on blood pressure and heart rate, compared with effects of vehicle injection, and to assess significant differences in the depressor responses to dopamine in the presence and absence of sulpiride.

## Results

*B-HT 958-induced hypotension and bradycardia*

In the preliminary dose-response studies, B-HT 958 62.5 to 2000 μg kg$^{-1}$ i.v. produced dose-dependent falls in mean arterial pressure and heart rate (Table 1) without an initial increase in blood pressure. Responses to B-HT 958 were maximal at a cumulative i.v. dose of 500 μg kg$^{-1}$.

In the i.v. control group, B-HT 958 350 μg kg$^{-1}$

**Table 1** Reductions in mean arterial pressure (MAP), heart rate (HR) and plasma noradrenaline concentration in response to cumulative i.v. doses of B-HT 958

| | | *ΔMAP* (mmHg) | *ΔHR* (beats $min^{-1}$) | *Plasma noradrenaline* ($ng\,ml^{-1}$) |
|---|---|---|---|---|
| Saline ($1\,ml\,kg^{-1}$ i.v.) | | + 1.2 ± 3.9 | + 4.5 ± 4.5 | 0.268 ± 0.02 |
| B-HT 958 ($\mu g\,kg^{-1}$ i.v.) | 62.5 | − 11.8 ± 3.9 | − 22.5 ± 6.7 | 0.244 ± 0.028 |
| | 125 | − 18.3 ± 3.1 | − 46.5 ± 2.9 | 0.197 ± 0.019 |
| | 250 | − 27.8 ± 5.2 | − 60 ± 3.5 | 0.186 ± 0.016 |
| | 500 | − 36.6 ± 7.5 | − 75 ± 9.3 | 0.169 ± 0.009 |
| | 1000 | − 33.5 ± 7.1 | − 76.5 ± 10.2 | 0.157 ± 0.017 |
| | 2000 | − 33 ± 6.1 | − 78 ± 8.8 | 0.167 ± 0.027 |

Changes in MAP and HR were measured 10 min after administration of saline and of each dose of B-HT 958. Plasma noradrenaline concentration was measured 5 min after each dose. Each value is the mean ± s.e.mean of 5 observations. Baseline values of mean arterial pressure and heart rate were 112.4 ± 3.9 mmHg and 397.2 ± 13.6 beats $min^{-1}$ respectively.

caused maximum falls in mean arterial pressure and heart rate of 31.3 ± 5.2 mmHg and 59 ± 12.9 beats $min^{-1}$ respectively ($P < 0.05$ in each case compared with pre-dose values, $n = 6$), 5 min after dosing. Blood pressure and heart rate did not recover to predose levels in the 60 min experimental period following administration of B-HT 958 (Figure 1). In the control group receiving saline 10 µl i.c.v., B-HT 958 caused falls in mean arterial pressure and heart rate of 27.2 ± 3.9 mmHg and 45 ± 4.3 beats $min^{-1}$ respectively ($P < 0.05$ in each case compared with pre-dose values, $n = 6$) 5 min after dosing. For the purpose of statistical analysis, the effects of antagonist pretreatment on the hypotensive and bradycardiac responses to B-HT 958 were determined 5 min after administration of the agonist, at the time of maximal effects in control animals.

B-HT 958-induced falls in mean arterial pressure and heart rate were significantly inhibited by pretreatment with sulpiride, 300 $\mu g\,kg^{-1}$ i.v. and 10 µg i.c.v., but not by pretreatment with i.v. or i.c.v. idazoxan (Figures 2 and 3). Sulpiride 50 µg i.c.v. did not cause further significant inhibition of B-HT 958-induced hypotension and bradycardia compared with the 10 µg i.c.v. dose (Figure 3). A combination of idazoxan 1000 $\mu g\,kg^{-1}$ and sulpiride 300 $\mu g\,kg^{-1}$ i.v. was also effective in antagonizing B-HT 958-induced falls in mean arterial pressure and heart rate, but did not cause significantly greater antagonism of the blood pressure and heart rate lowering effects of B-HT 958 than sulpiride given alone (Figure 2). The combination of idazoxan and sulpiride had a significantly greater effect in inhibiting B-HT 958 hypotension than idazoxan 1 $mg\,kg^{-1}$ alone, the latter causing slight but non significant reduction of the fall in mean arterial pressure.

During the 5 min infusion of idazoxan 1000 $\mu g\,kg^{-1}$ plus sulpiride 300 $\mu g\,kg^{-1}$ i.v., mean arterial pressure

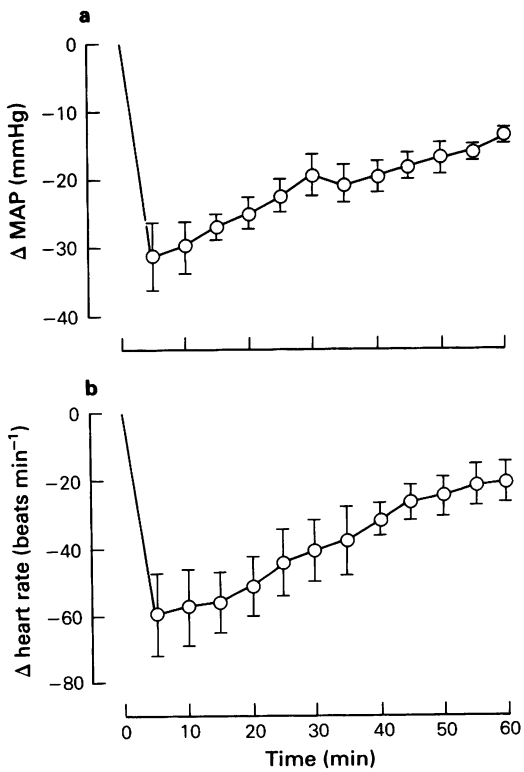


**Figure 1** Time course of the hypotensive and bradycardiac response to B-HT 958 350 $\mu g\,kg^{-1}$ i.v. B-HT 958 was given at $t = 0$ min. (a) Decreases in mean arterial pressure (ΔMAP) and (b) decreases in heart rate (Δheart rate) from baseline values of 113.2 ± 3.1 mmHg and 370 ± 16.4 beats $min^{-1}$ respectively, produced by B-HT 958 over 60 min. Each point is the mean of 6 observations with vertical lines indicating s.e.mean.

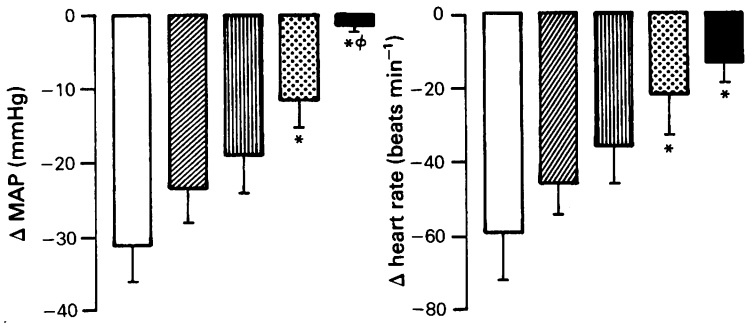


**Figure 2** The effect of i.v. administration of idazoxan or sulpiride on the decrease in mean arterial pressure (ΔMAP) and heart rate produced by B-HT 958 350 μg kg$^{-1}$ i.v. Falls in mean arterial pressure and heart rate were measured 5 min after administration of B-HT 958, at the time of maximal responses in control animals. (□) vehicle pretreatment; (▨) idazoxan 300 μg kg$^{-1}$; (▩) idazoxan 1000 μg kg$^{-1}$; (▦) sulpiride 300 μg kg$^{-1}$; (■) idazoxan 1000μgkg$^{-1}$ + sulpiride 300 μg kg$^{-1}$. Each histobar is the mean of 6 observations with vertical lines indicating s.e.mean. *$P < 0.05$ compared with vehicle; (Ø) $P < 0.05$ compared with idazoxan 1000 μg kg$^{-1}$. Mean arterial pressure prior to administration of B-HT 958 was 113.3 ± 3.1, 120 ± 5.1, 110.3 ± 5.9, 115.2 ± 2.7 and 120 ± 3.4 mmHg in the control, idazoxan 300 μg kg$^{-1}$, idazoxan 1000 μg kg$^{-1}$, sulpiride 300 μg kg$^{-1}$ and idazoxan 1000 μg kg$^{-1}$ + sulpiride 300 μg kg$^{-1}$ pretreated groups respectively. Corresponding values for heart rate were 370 ± 16.4, 428 ± 18.2, 431 ± 2.2, 383 ± 4.2 and 428 ± 10.4 beats min$^{-1}$.

rose by 16.17 ± 2.43 mmHg ($n = 6$). This increase in pressure was significantly greater ($P < 0.05$) than that observed during infusion of saline vehicle (1.7 ± 0.6 mmHg). Idazoxan i.v. alone, i.c.v. idazoxan, i.v. and i.c.v. sulpiride had no significant effects on resting blood pressure.

Heart rate rose by 52.0 ± 7.5, 48.0 ± 12.8 and 37.0 ± 4.2 beats min$^{-1}$ during i.v. infusion of idazoxan 300 μg kg, 1000 μg kg$^{-1}$ and idazoxan 1000 μg kg$^{-1}$ plus sulpiride 300 μg kg$^{-1}$ respectively ($P < 0.05$ in each case $n = 6$). Intracerebroventricular idazoxan, i.v. and i.c.v. sulpiride did not significantly affect resting heart rate.

In control experiments to determine the effects of antagonists alone on blood pressure and heart rate, saline 1 ml kg$^{-1}$ i.v. was given 5 min after infusion of the antagonists. Mean arterial pressure did not change significantly from predose levels 5 min after administration of saline in animals pretreated with idazoxan 300 μg kg$^{-1}$ i.v. or 20 μg i.c.v., and sulpiride 300 μg kg$^{-1}$ i.v. or 10 μg i.c.v. Similarly, heart rate did not change significantly from pre-saline values, except

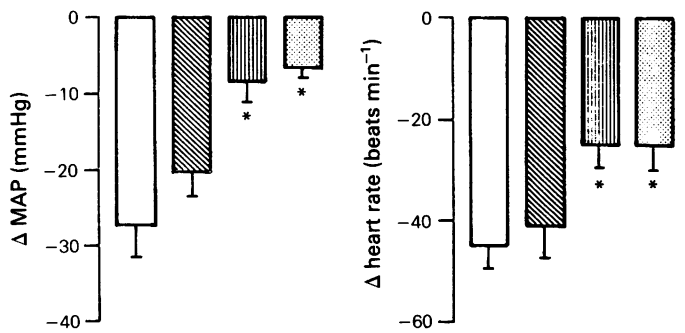


**Figure 3** The effect of i.c.v. administration of idazoxan or sulpiride on the decrease in mean arterial pressure (ΔMAP) and heart rate produced by B-HT 958 350 μg kg$^{-1}$ i.v. Falls in mean arterial pressure and heart rate were measured 5 min after administration of B-HT 958, at the time of maximal responses in control animals. (□) vehicle pretreatment; (▨) idazoxan 20 μg i.c.v.; (▩) sulpiride 10 μg i.c.v.; (▦) sulpiride 50 μg i.c.v. Each histobar is the mean of 6 observations with vertical lines indicating s.e.mean. *$P < 0.05$ compared with vehicle pretreatment. Mean arterial pressure prior to administration of B-HT 958 was 118.2 ± 5.6, 119.3 ± 6.2, 120.8 ± 2.8 and 122 ± 4.2 mmHg in the control, idazoxan 20 μg, sulpiride 10 μg and sulpiride 50 μg pretreated groups respectively. Corresponding values for heart rate were 377 ± 11.6, 405 ± 15, 380 ± 7.7 and 384 ± 9.8 beats min$^{-1}$.

**Table 2** Effect of i.v. administration of idazoxan and sulpiride on the plasma noradrenaline response to B-HT 958

| *Experimental group* | *Time* (min) − 15 | − 5 | + 5 | + 10 |
|---|---|---|---|---|
| Control (saline 1 ml $kg^{-1}$) | 0.189 ± 0.02 | 0.187 ± 0.03 | 0.121 ± 0.02 | 0.110 ± 0.02 |
| Idazoxan (300 $\mu g\ kg^{-1}$) | 0.163 ± 0.02 | 0.466 ± 0.06 | 0.336 ± 0.05 | 0.342 ± 0.06 |
| Idazoxan (1 mg $kg^{-1}$) | 0.200 ± 0.03 | 0.529 ± 0.03 | 0.314 ± 0.04 | 0.367 ± 0.04 |
| Sulpiride (300 $\mu g\ kg^{-1}$) | 0.238 ± 0.02 | 0.235 ± 0.02 | 0.204 ± 0.02 | 0.198 ± 0.01 |
| Sulpiride (300 $\mu g\ kg^{-1}$) + idazoxan (1 mg $kg^{-1}$) | 0.200 ± 0.02 | 0.429 ± 0.08 | 0.382 ± 0.07 | 0.378 ± 0.08 |

Idazoxan and/or sulpiride were given as a 5 min infusion from − 10 to − 5 min. B-HT 958 350 $\mu g\ kg^{-1}$ i.v. was given at $t = 0$ min. Plasma noradrenaline levels are expressed in ng $ml^{-1}$. Each value is the mean ± s.e.mean of 6 observations. The significance of the effects of antagonist pretreatment are indicated in the text.

in the group receiving idazoxan 300 $\mu g\ kg^{-1}$ i.v. where a fall of − 17 ± 6.5 beats $min^{-1}$ ($P < 0.05$) was recorded.

*Plasma noradrenaline*

In the dose-response studies, B-HT 958 62.5–2000 $\mu g\ kg^{-1}$ i.v. caused reductions in plasma noradrenaline concentration which were maximal after a cumulative i.v. dose of 1000 $\mu g\ kg^{-1}$ (Table 1). In both sets of control animals receiving B-HT 958 350 $\mu g\ kg^{-1}$, the hypotensive response was associated with significant reductions in plasma noradrenaline concentration of 35–40% 5 and 10 min after administration (Tables 2 and 3, $P < 0.05$ in each case, ONEWAY). The plasma noradrenaline levels in samples taken 30 min and 60 min after B-HT 958 were not significantly different from pre-B-HT 958 levels (results not shown). Therefore for the purpose of statistical analysis the effects of antagonist treatment on the reduction in plasma noradrenaline concentration produced by B-HT 958 was assessed from 15 min before administration of B-HT 958 to 10 min after its administration. Idazoxan 300 $\mu g\ kg^{-1}$ i.v. and 1000 $\mu g\ kg^{-1}$ i.v. caused immediate, large rises in plasma noradrenaline to 300 ± 36 and 285 ± 32% of basal values respectively (Table 2). Neither i.v. dose of idazoxan significantly inhibited the fall in plasma noradrenaline concentration caused by B-HT 958 (Table 2). When idazoxan 300 $\mu g\ kg^{-1}$ i.v. was given followed by saline in place of B-HT 958, plasma noradrenaline concentration was increased as before (Table 4). Plasma noradrenaline levels remained elevated 5 and 10 min after i.v. saline and were not significantly different from pre-saline (− 5 min) values.

Idazoxan, 20 $\mu g$ i.c.v., produced a rise in plasma noradrenaline to 140 ± 9.6% of the basal value, and

**Table 3** Effect of i.c.v. administration of idazoxan and sulpiride on the plasma noradrenaline response to B-HT 958

| *Experimental group* | *Time* (min) − 15 | − 5 | + 5 | + 10 |
|---|---|---|---|---|
| Control (saline 10 $\mu l$ i.c.v.) | 0.222 ± 0.02 | 0.254 ± 0.02 | 0.156 ± 0.01 | 0.166 ± 0.02 |
| Idazoxan (20 $\mu g$ i.c.v.) | 0.275 ± 0.02 | 0.376 ± 0.03 | 0.251 ± 0.02 | 0.267 ± 0.02 |
| Sulpiride (10 $\mu g$ i.c.v.) | 0.193 ± 0.02 | 0.207 ± 0.02 | 0.183 ± 0.02 | 0.184 ± 0.01 |
| Sulpiride (50 $\mu g$ i.c.v.) | 0.251 ± 0.02 | 0.245 ± 0.02 | 0.246 ± 0.03 | 0.222 ± 0.02 |

Idazoxan or sulpiride were given as a 5 min infusion from − 10 to − 5 min (2 $\mu l\ min^{-1}$). B-HT 958 350 $\mu g\ kg^{-1}$ i.v. was given at $t = 0$ min. Plasma noradrenaline levels are expressed in ng $ml^{-1}$. Each value is the mean ± s.e.mean of 6 observations. The significance of the effects of antagonist pretreatment are indicated in the text.

**Table 4** The effect of i.v. and i.c.v. administration of idazoxan and sulpiride on plasma noradrenaline concentration in the absence of B-HT 958

| *Experimental group* | *Time* (min) | | | | |
|---|---|---|---|---|---|
| | − 15 | − 5 | + 5 | + 10 | *P* value (ONEWAY) |
| Idazoxan ($300\,\mu g\,kg^{-1}$ i.v.) | 0.396 ± 0.04 | 0.795 ± 0.09 | 0.711 ± 0.06 | 0.671 ± 0.07 | $P<0.01$ |
| Idazoxan ($20\,\mu g$ i.c.v.) | 0.413 ± 0.05 | 0.514 ± 0.06 | 0.548 ± 0.06 | 0.628 ± 0.05 | $P<0.01$ |
| Sulpiride ($300\,\mu g\,kg^{-1}$) | 0.213 ± 0.02 | 0.243 ± 0.03 | 0.218 ± 0.01 | 0.222 ± 0.03 | NS |
| Sulpiride ($10\,\mu g$ i.c.v.) | 0.255 ± 0.02 | 0.254 ± 0.02 | 0.248 ± 0.02 | 0.220 ± 0.02 | NS |

Idazoxan or sulpiride were given as a 5 min infusion from − 10 to − 5 min. Saline 1 ml $kg^{-1}$ i.v. was given at $t = 0$ min. Plasma noradrenaline levels are expressed in ng $ml^{-1}$. Each value is the mean ± s.e.mean of 6 observations. The *P* value indicates the degree of variation in plasma noradrenaline concentration with time in each treatment group. NS: not significant.

did not attenuate the B-HT 958-induced fall in plasma noradrenaline ($P > 0.05$ TWOWAY, Table 3). When B-HT 958 was not given, this i.c.v. dose of idazoxan caused a gradual significant rise in plasma noradrenaline concentration (Table 4). These effects of idazoxan on plasma noradrenaline concentration are unlikely to be due to blood loss, as we have previously shown that repeated sampling from anaesthetized rats over this time course has no significant effects on plasma noradrenaline concentration (Brown & Harland, 1984).

Sulpiride $300\,\mu g\,kg^{-1}$ i.v. and $10\,\mu g$ and $50\,\mu g$ i.c.v. significantly inhibited the fall in plasma noradrenaline 5 and 10 min after B-HT 958 ($P < 0.05$ in each case TWOWAY, Tables 2 and 3). When saline 1 ml $kg^{-1}$ was given in place of B-HT 958, sulpiride $300\,\mu g\,kg^{-1}$ i.v. and $10\,\mu g$ i.c.v. had no significant effects on plasma noradrenaline concentration (Table 4).

The combination of idazoxan $1000\,\mu g\,kg^{-1}$ i.v. and sulpiride $300\,\mu g\,kg^{-1}$ i.v. caused a rise in plasma noradrenaline to 203 ± 34.8% of the basal value (Table 2). This combination of antagonists almost completely blocked the fall in plasma noradrenaline concentration caused by B-HT 958 $350\,\mu g\,kg^{-1}$ i.v.

*Effect of sulpiride on depressor responses to dopamine*

In animals treated with propranolol 1 mg $kg^{-1}$ i.v. and phentolamine 5 mg $kg^{-1}$ i.v., dopamine 25, 50 and $100\,\mu g\,kg^{-1}$ i.v. caused a fall in mean arterial pressure (Table 5). Sulpiride $300\,\mu g\,kg^{-1}$ i.v. significantly reduced the depressor effects of each dose of i.v. dopamine, whereas sulpiride $50\,\mu g$ i.c.v. was without effect (Table 2).

**Table 5** Effect of central and peripheral administration of sulpiride on the changes in mean arterial pressure produced by dopamine

| *Experimental group* | *Dopamine* ($\mu g\,kg^{-1}$ i.v.) | | |
|---|---|---|---|
| | 25 | 50 | 100 |
| Controls | − 8.5 ± 1.2 | − 10.75 ± 1.9 | − 12.5 ± 1.3 |
| Sulpiride ($300\,\mu g\,kg^{-1}$ i.v.) | − 5.5 ± 0.3* | − 5 ± 0.4* | − 5.25 ± 0.75** |
| Sulpiride ($50\,\mu g$ i.c.v.) | − 9.25 ± 0.75 | − 11.75 ± 1.3 | − 10 ± 0.8 |

All animals were treated with propranolol 1 mg $kg^{-1}$ i.v. and phentolamine 5 mg $kg^{-1}$ i.v. Changes in mean arterial pressure are expressed in mmHg. Each value is the mean ± s.e.mean, $n = 4$.
*$P < 0.05$; **$P < 0.01$ compared with controls (Bonferroni method). Mean arterial pressure prior to dopamine administration was 68 ± 3.3 mmHg, 78.5 ± 4.3 mmHg and 79.75 ± 4.2 mmHg in the control, i.v. sulpiride and i.c.v. sulpiride pretreated groups respectively.

## Discussion

In this study we have tested the hypothesis that the hypotensive and bradycardiac effects of B-HT 958 are due to its agonist action at peripheral prejunctional $\alpha_2$-adrenoceptors. We have attempted to block the actions of B-HT 958 with central and peripheral administration of the selective $\alpha_2$-adrenoceptor antagonist, idazoxan, at doses previously shown to be effective against clonidine-induced hypotension and bradycardia in the anaesthetized rat (Berridge *et al.*, 1982; Brown & Harland, 1984).

B-HT 958 produced marked reductions in mean arterial pressure and heart rate in anaesthetized rats, confirming the results of Kobinger & Pichler (1984). The failure of i.c.v. idazoxan to attenuate B-HT 958-induced falls in blood pressure, heart rate and plasma noradrenaline concentration confirmed that at least in anaesthetized rats, the cardiovascular effects of B-HT 958 are not due to stimulation of central $\alpha_2$-adrenoceptors. However, the results of the present study suggest that the cardiovascular effects of B-HT 958 are also not due to stimulation of peripherally located $\alpha_2$-adrenoceptors, as idazoxan at doses up to 1 mg $kg^{-1}$ i.v. did not significantly inhibit B-HT 958-induced falls in blood pressure, heart rate or plasma noradrenaline concentration. The failure of i.v. idazoxan to attenuate the B-HT 958-induced fall in plasma noradrenaline concentration could be due to the fact that the antagonist itself increased plasma noradrenaline almost three fold. This seems unlikely however as idazoxan at 300 $\mu g\ kg^{-1}$ effectively inhibits the reduction in plasma concentrations of noradrenaline and adrenaline produced by i.v. clonidine, despite the large rises in plasma catecholamines produced by the antagonist itself (Brown & Harland, 1984). In addition, such a rise in plasma noradrenaline concentration was seen in the present study when idazoxan and sulpiride were given together, but this combination of antagonists significantly inhibited the B-HT 958-induced fall in plasma noradrenaline. Such large increases in plasma noradrenaline concentration are unlikely to be due to activation of cardiovascular reflexes such as the baroreceptor reflex, as idazoxan does not decrease arterial pressure in anaesthetized rats. In fact it tends to cause a small increase in some animals which is probably due to a partial agonist activity at $\alpha$-adrenoceptors (Hannah *et al.*, 1983; Limberger & Starke, 1983). Alternatively, this rise in blood pressure could be a consequence of the increase in plasma noradrenaline caused by idazoxan. It must also be noted that when idazoxan was given i.v. in the absence of B-HT 958, the increase in plasma noradrenaline was sustained at the times when B-HT 958, if given, would have caused a reduction.

B-HT 920 is an azepine derivative chemically related to B-HT 958 and considered to be a selective $\alpha_2$-adrenoceptor agonist (Hammer *et al.*, 1980; Kobinger & Pichler, 1981; van Meel *et al.*, 1981). This compound was recently shown to be a potent agonist at prejunctional dopamine receptors in the central and peripheral nervous systems (Anden *et al.*, 1982; 1983; Willfert *et al.*, 1984). Numerous studies have shown that certain dopamine receptor agonists such as lergotrile, N, N-di-n-propyldopamine (DPDA) and pergolide reduce blood pressure in conscious spontaneously hypertensive rats and a number of anaesthetized animal preparations (for review see Cavero *et al.*, 1982b). These cardiovascular effects are thought to be due to stimulation of $DA_2$-dopamine receptors at prejunctional sites on axonal varicosities of postganglionic sympathetic nerves (Cavero *et al.*, 1982a), as they can be antagonized by drugs which preferentially block the $DA_2$-dopamine receptor, such as S-sulpiride RS-sulpiride and haloperidol (Barrett & Lockhandwala, 1981; Cavero *et al.*, 1982b). As we have found that the reduction in blood pressure and heart rate produced by B-HT 958 can be antagonized by i.v. sulpiride at a dose which shows no $\alpha_2$-adrenoceptor blocking activity in the rat (Willfert *et al.*, 1984), it appears that stimulation of $DA_2$-dopamine receptors is the main mode of action by which B-HT 958 produces these effects in anaesthetized rats. As the reduction in plasma noradrenaline concentration produced by B-HT 958 can be attenuated by blockade of dopamine receptors, it is possible that the reduction of plasma noradrenaline reflects stimulation of $DA_2$-dopamine receptors on postganglionic sympathetic nerve varicosities or on sympathetic ganglia by B-HT 958. However, sulpiride 10 $\mu g$ i.c.v. caused approximately the same inhibition of the cardiovascular depressant actions of B-HT 958 as i.v. sulpiride, suggesting that these effects are due to changes in sympathetic activity secondary to activation of dopamine receptors within the central nervous system, rather than stimulation of peripheral $DA_2$-dopamine receptors. This is most probably the case with respect to B-HT 958-induced bradycardia, as there is considerable evidence that the cardiac sympathetic neurones of the rat do not possess prejunctional dopamine receptors (Hicks & Cannon, 1979; Cavero *et al.*, 1981b; Willfert *et al.*, 1984).

There is some evidence that the $DA_2$-dopamine receptor agonist pergolide can inhibit peripheral sympathetic nerve activity by stimulation of central dopamine receptors (Barrett & Lockhandwala, 1982; Jadhav *et al.*, 1983). Additionally in the anaesthetized rat, intracisternal (i.c.i.) or i.c.v. pergolide produced the same fall in blood pressure and heart rate as an equivalent i.v. dose. It may be, however, that the central dopamine receptors stimulated by pergolide are not protected by an adequate blood–brain barrier, as the hypotensive effect of i.c.i. or i.c.v. pergolide can be blocked by i.v. domperidone (Cavero *et al.*, 1984),

at a dose not thought to cross the blood–brain barrier easily (Sved & Fernstrom, 1980).

Since we completed our studies, it has been reported that B-HT 958 stimulates dopamine autoreceptors in mouse and rat brain (Grabowska-Anden & Anden, 1984; Hortnagl *et al.*, 1984). No prejunctional $\alpha_2$-adrenoceptor agonist action was observed for B-HT 958 in these test systems, although higher doses were found to antagonise the prejunctional effects of clonidine (Grabowska-Anden & Anden, 1984), confirming the $\alpha_2$-adrenoceptor antagonist properties of the drug.

It is possible that some of the prejunctional effects of B-HT 958 in the rat heart which were antagonized by yohimbine and attributed to stimulation of prejunctional $\alpha_2$-adrenoceptors (Pichler *et al.*, 1982), could have been due to prejunctional dopamine receptor stimulation, as yohimbine possesses some dopamine receptor blocking properties (Scatton *et al.*, 1980). In addition, the lack of vasoconstrictor activity of B-HT 958 *in vivo*, at doses of the same order as reduce release of noradrenaline *in vitro*, may be explained not by its selective agonist action at prejunctional rather than postjunctional $\alpha_2$-adrenoceptors, but by its preferential stimulation of cardiovascular dopamine receptors.

The failure of the highest i.c.v. dose of sulpiride used in this study to reduce the depressor effect of dopamine in $\alpha$- and $\beta$-adrenoceptor blocked rats, indicates that there was no significant leakage of this dose from the brain into the peripheral circulation

This fall in blood pressure in response to dopamine is thought to be due to stimulation of postjunctional $DA_1$-dopamine receptors. It is possible that $DA_2$-dopamine receptors are involved in this response in the rat, as it can be effectively reduced by both i.v. sulpiride (this study) and haloperidol (Chevillard & Mathieu, 1979), both preferential $DA_2$-dopamine receptor antagonists. However, RS-sulpiride is less selective for the $DA_2$-dopamine receptor than S-sulpiride (Horn *et al.*, 1980; Llenas *et al.*, 1982) and may cause some $DA_1$-dopamine receptor blockade. Racemic sulpiride is an effective antagonist of dopamine-induced increases in renal and mesenteric blood flow in the anaesthetized dog (Clark & Menninger, 1980; Drew & Hilditch, 1980; Llenas *et al.*, 1982), despite having a low affinity for $DA_1$-dopamine receptors *in vitro* (Schmidt *et al.*, 1981; Hilditch & Drew, 1981).

In conclusion, the results of the present study suggest that in anaesthetized rats, the hypotensive and bradycardiac effects of B-HT 958 cannot be attributed to its agonist action at peripheral prejunctional $\alpha_2$-adrenoceptors and are due rather to stimulation of dopamine receptors probably located within the central nervous system.

D.H. is an M.R.C. scholar. M.J.B. is an M.R.C. Senior Fellow. We would like to thank Mr V. Aber (Statistician, Royal Postgraduate Medical School) for analysing the data.

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