Pyridazine Derivatives. XI: Antihypertensive Activity of 3-Hydrazinocycloheptyl[1,2-c]pyridazine and Its Hydrazone Derivatives

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Abstract □ 3-Hydrazinocycloheptyl[1,2-c]pyridazine (4) and its hydrazone derivatives 3-{N-(iso-propylidene)}hydrazinocycloheptyl[1,2-c]pyridazine (5) and 3-{N-(isobutylidene)}hydrazinocycloheptyl[1,2-c]pyridazine (6) were prepared, and their activity against genetic, neurogenically-induced, and deoxycorticosterone acetate (NaCl)-induced hypertension was found to be at least as great as that of hydrazine. The results of studying vasorelaxation of rat aorta by 4 and hydrazine suggest that both these compounds owe their antihypertensive activity to direct relaxation of vascular smooth muscle.

Hydrazinophthalazines were first synthesized by Gross et al.1 in 1950 and are used clinically as antihypertensives, the most important being hydrazine and dihydrazine. Structure—activity studies have shown significant antihypertensive activity to be exhibited by a number of related hydrazine pyridazines, and this group of compounds continues to be the object of prospect for new drugs with greater activity and fewer side effects. For several years we have been working on the synthesis of modified hydrazine pyridazines, most of which have proved to have considerable antihypertensive activity.

We now report the results of a detailed study of the antihypertensive activity of 3-hydrazinocycloheptyl[1,2-c]pyridazine (4) and its hydrazone derivatives, 3-{N-(iso-propylidene)}hydrazinocycloheptyl[1,2-c]pyridazine (5) and 3-{N-(isobutylidene)}hydrazinocycloheptyl[1,2-c]pyridazine (6), together with a modified synthetic procedure.

Experimental Section

Chemistry—Melting points were determined with a Gallenkamp melting point apparatus and were uncorrected. IR spectra were recorded with a Perkin-Elmer 260 FT (Fourier transform) spectrophotometer. H1 NMR spectra were recorded with a Bruker WM 250 spectrophotometer using tetramethylsilane as internal standard. Microanalysis was performed with a Perkin-Elmer 240 B instrument in the Microanalysis Service of the University of Santiago de Compostela; results were within ±0.4% of the theoretical values.

3-Chlorocycloheptyl[1,2-c]pyridazine (3)—Cycloheptyl[1,2-c]3(2H)pyridazinone (2; 2 g, 0.0066 mol) was dissolved in tetrachloroethane (50 mL) by heating. Chlorosulphonyl isocyanate (0.16 g, 0.0066 mol) to tetrachloroethane (15 mL) was added, and the resulting solution was warmed at 60 °C with stirring for 6 h. The reaction mixture was then left to stand overnight at room temperature, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel, with chloroform-methanol as eluant, to afford 0.44 g (40%) of a yellow liquid that was used in the next stage without further purification; IR (NaCl): 3100–2850 (NH); 1600 cm = C amn; 750–640 cm =; no cyanobenzane bands were present.

3-Hydrazinocycloheptyl[1,2-c]pyridazine (4)—A suspension of 2 g (0.012 mol) of 3 in 20 mL of 41 mol% of 98% hydrazine hydrate was refluxed for 2 h and then cooled to obtain a solid that on recrystal-
solution containing 0.5 mM ethylene glycol-bis(β-aminoethyl ether)-
N,N‘,N′,N′-tetraacetic acid (EGTA) before addition of NA. To study the
effects of hydralazine and 4, the preparations were further washed in
normal Krebs solution for 60 min to fill the Ca²⁺ stores depleted
by the first contraction, and preincubated for a further 20 min in
calcium-free solution before a suitable concentration of hydralazine
or 4 was added, followed 10 min later by NA. Other aorta rings were
simultaneously subjected to the same procedure, but omitting 4 and
hydralazine.

⁴⁰Ca Influx—Aortic rings weighing 5–9 mg were equilibrated for at
least 60 min in physiological solution (composition (mM): NaCl (139),
KCl (5), MgCl₂ (1), CaCl₂ (1.5), N₂-hydroxyethylpiperazine-N₂-
ethanesulfonic acid (HEPES) (5) glucose (10)] maintained at 37°C
and aerated with 100% O₂. The rings were then incubated for 5 min in
a medium containing 0.6 μCi mL⁻¹⁴⁰Ca (New England Nuclear,
Madrid, Spain; specific activity 35 mCi mL⁻¹ mg⁻¹) with or without 10 μM
NA or 60 mM K⁺. To investigate the effects of hydralazine and 4 on
the uptake, these agents were added to the equilibration medium 20
min before incubation and also to the incubation medium. After
incubation, the rings were washed for 45 min in 500 mL of an ice-cold
calcium-free physiological solution of pH 7.4 containing 2 mM EGTA,
which was bubbled with 100% O₂ to remove extracellular Ca²⁺ from
the tissue. The rings were then blotted, weighed, and digested in 1 mL
H₂O₂ (110 volumes) at 115°C for 90 min. After cooling, 5 mL of
Resin-Solubilant mixture were added and the radioactivity of the samples
was measured in a liquid scintillation counter (Beckman LS 3801).
Expression and Statistical Analysis of Results—Unless otherwise
specified, results shown in the text and figures are means ± standard
events of the mean (SEM). The statistical significance of differences
between means was estimated by the Student’s two-tailed t test for
paired or unpaired data.

Antihypertensive activity and effects on heart rate were quantified
as percentage change with respect to baseline values. The ED₉₀ (the
dosage necessary to produce a 30% fall in SAP or a 30% rise in heart
rate) was calculated from the equations of straight lines fitted to the
response-log dosage data.

The ⁴⁰Ca uptake was calculated as follows: ⁴⁰Ca uptake (nmol
⁴⁰Ca/kg wet tissue) = [counts per min in tissue/wet tissue weight (kg)]
× [mg ⁴⁰Ca in 1 L solution counts per min in 1 L of solution]. To
avoid inaccuracy in the calculation of ⁴⁰Ca uptake, the 1.5 mM Ca²⁺
present in physiological solution was not included in the "nmol ⁴⁰Ca
in 1 L of solution" factor of this expression.

The 50% inhibition concentrations (IC₅₀) of hydralazine and 4 were
calculated from their cumulative dose-response curves.

Drugs and Chemicals—The following drugs were used: (+)-noradren-
aline bitartrate, atrazine sulfate, and DOCA (Sigma, Madrid, Spain);
hydralazine hydrochloride (Giba Geigy, Barcelona, Spain); and com-
ounds 4, 5, and 6. Hydralazine, 4, 5, 6, and atripine solutions were
dissolved in deionized water immediately before use. Microsuspen-
sion of DOCA (1%) was prepared in a 0.25% polysorbate 80 (Tween
80; 0.125% carboxymethyl cellulose, and 0.9% of NaCl. NA was prepared
with deionized water from a 100 mM stock solution kept at -20°C; 0.2% sodium
bentonite was added to prevent oxidation.

The chemicals used in the preparation of the physiological solutions
were of analytical grade.

Results and Discussion

Chemistry—Compound 4 was previously prepared by a
general four-step procedure: (1) synthesis of the morpholin-
ium salt of cycloheptane-1, 2; (2) reaction with hydrazine
hydrate to give cyclohexyl-1,2-cyclohexylidene) pyridazine, 2; (3)
chlorination with phosphorus oxychloride; and (4) introduc-
tion of the NO₂ group to give compound 41. The limiting step of this pro-
dure is step 3, which has yields of 20% or less. We have now
found that these yields can be increased to 40–50% with
the recently described chlorinating agent* chlorosulfonyl iso-
cyanate (Scheme 1).

The hydrazones 3-(N²-isopropylidene)hydrazinocyclo-
heptylidene)hydrazinocyclohexylidene)pyridazine 5 and 3-(N²-isobutylidene)hydrazin-
ocyclohexylidene)pyridazine 6 were prepared from 4 as
described by Rovina and Estevêz.

Pharmacology—Antihypertensive Effects—Antihypertensive
activity was evaluated for hypertension of three different
origins. The new compounds tested clearly reduced SAP

(1) and increased heart rate (Table I) in SHR 1 h after
administration. At a dosage level of 5 mg/kg, the percentage
reductions in SAP induced by 4 (40.24 ± 1.1%) and 6 (38.29 ±
1.5%) were significantly greater (p < 0.05) than that
induced by hydralazine (33.53 ± 1.15%). At other dosage
levels, the differences were not statistically significant and
either were the differences in percentage increase in heart
rate (at any dosage level). As shown in Tables I and II, the new
compounds were clearly more effective than hydralazine in
reducing both neurogenic- and DOCA-NaCl-induced hy-
pertension 1 h after administration. There were no significant
variations in either SAP or heart rate following control
administration of deionized water.

Vasorelaxation in Normal Krebs Solution—NA (10 μM)
and high potassium concentration (80 mM) produced sustained
contractions of 4137 ± 402 and 3239 ± 304 mg (n = 10,
respectively, in isolated rat aorta arterial rings. These
contractions were considered the maximum response (100%).

Hydralazine (0.1–5 mM) and 4 dose dependently and non-
specifically inhibited the contractions induced by NA (IC₅₀ =
0.68 ± 0.019 and 2.1 ± 0.16 mM, respectively) and by K⁺
(IC₅₀ = 0.6 ± 0.064 and 4.2 ± 0.28 mM, respectively; Figure
1). For neither hydralazine nor 4 was there any significant
difference between the IC₅₀ values obtained with K⁺ and NA
(n = 5, p > 0.05; Figure 2).

Vasorelaxation in Calcium-Free Krebs Solution—NA (10
μM) produced its characteristic two-component contraction;
that is, an initial transient (fast component) tension of 1125
± 51 mg (n = 10) relaxing to a sustained (slow component)
tension of 270 ± 24 mg. Both phases were strongly inhibited
by 1 mM hydralazine, which reduced the transient tension to
95.6 ± 8 mg and the sustained tension to 40 ± 5 mg (n = 5,
p < 0.01), and by 4, which reduced the transient tension to
227 ± 16 mg and the sustained tension to 91 ± 9 mg (n = 5, p <
0.01).

⁴⁰Ca Uptake—Calcium uptake by the rat aorta segments in
the absence of other agents (basal uptake) was 9.0 ± 0.21
nmol kg⁻¹ mg⁻¹ (n = 10). The addition of 4 or hydralazine (1 mM)
did not affect the uptake significantly (tissue ⁴⁰Ca contents

Table I—Effects of SAP

<table>
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<th>SHR</th>
<th>NHR</th>
<th>DOCA-NaCl Rats</th>
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<tr>
<td></td>
<td>ED₉₀ (mg/kg)</td>
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<td>Hydralazine</td>
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<td>4.34</td>
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significantly inhibited NA- and K+-induced $^{45}$Ca uptake (tissue $^{45}$Ca contents for 4, $12.32 \pm 0.64$ nmol·kg$^{-1}$ with NA and $14.1 \pm 0.59$ nmol·kg$^{-1}$ with 60 mM K$^+$; $n = 5$, $p > 0.05$). For hydralazine, $11.31 \pm 0.82$ nmol·kg$^{-1}$ with NA and $13.4 \pm 0.72$ nmol·kg$^{-1}$ with 60 mM K$^+$ ($n = 5$, $p > 0.05$; Figure 3).

The above results show that the hypotensive activities of both hydralazine and the new compounds against all three types of hypertension considered are greater than those in normotensive rats. In general, the new compounds were at least as active as hydralazine.

The type of hypertension least affected by the new compounds was that of genetic origin, which is characterized, after onset, by normal cardiac output and increased peripheral resistance. It is the type that is most similar to human essential hypertension; its development and maintenance involve a number of mechanisms that are still not fully understood and that may make it more refractory to pharmacological treatment than the others. The new compounds were more active than hydralazine against both hypertension induced by manipulation of the sympathetic system (by denervation), which also involves increased peripheral resistance, and against hypertension induced by DOCA-NaCl, which may involve hyperreactivity of the sympathetic system and/ or retention of sodium and water due to electrolyte imbalance.

The tachycardia that accompanies the fall in blood pressure induced by hydralazine in conscious normotensive rats has been reported as being a sympathetic reflex initiated by the detection of the drop in pressure by arterial baroreceptors, though the poor correlation between arterial pressure changes and heart rate in some studies has led to suggestions that a central mechanism or hemodynamic factors may be involved. The antihypertensive effects of the new compounds were accompanied by increased heart rate in both SHR and DOCA-NaCl rats, suggesting that the tachycardia was of reflex origin. However, in NHR, which lack the afferent pathways from arterial baroreceptors, a very wide range of heart rates was observed, with a less evident increase than for the other types of hypertension. This may have been because the cardiac stimulus was not totally reflex in nature, having a central contribution also, or because denervation was not complete, or because the fibers of vagus nerve baroreceptors took over control of the bulbar cardiovascular neurons.

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Table II—Effects on Heart Rate

<table>
<thead>
<tr>
<th>Compound</th>
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<th>DOCA-NaCl</th>
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<td>Hydralazine</td>
<td>&gt;5</td>
<td>—</td>
<td>&gt;10</td>
</tr>
<tr>
<td>4</td>
<td>&gt;5</td>
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<td>6</td>
<td>4.53</td>
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*a* — Not determined.

Figure 1—Effect of hydralazine (0.1−5 mM) on contractions of rat aorta rings without endothelium induced by K$^+$ (60 mM; ■) and NA (10 μM; ○). Each point represents the mean ± standard error of the mean (SEM) of five experiments. Key: (+) $p < 0.05$; (++) $p < 0.01$ with respect to the maximum tension (100%). The $IC_{50}$ values are 0.6 ± 0.064 and 0.68 ± 0.019 mM for NA and K$^+$, respectively.

Figure 2—Effect of C4 (0.1−5 mM) on contractions of rubbed rat aorta rings induced by K$^+$ (60 mM; ■) and NA (10 μM; ○). Data are plotted as mean ± SEM (n = 5). Key: (+) $p < 0.05$; (++) $p < 0.01$ with respect to the maximum tension (100%). The $IC_{50}$ values are 4.2 ± 0.28 and 2.1 ± 0.16 mM for NA and K$^+$, respectively.

Figure 3—Effects of C4 (1 mM) and hydralazine (HYD, 1 mM) on $^{45}$Ca uptake induced by K$^+$ (60 mM) and NA (10 μM) in rubbed rat aorta rings. Each bar shows the mean ± SEM of five experiments. Key: (+) $p < 0.05$; (++) $p < 0.01$ with respect to the basal uptake.
The hydrazones of hydralazine are generally unstable. Liberating parent drug into the blood. However, it is not yet clear whether the hypotensive effect of hydrazones of hydralazine should be attributed to their intrinsic activity or to back-conversion to the parent drug, hydralazine.28,29 The stability of hydrazones in vitro, even at pH 7.4, is not well understood. The literature contains conflicting results8,12,24 and in the present study we present in vitro data for 4 (the most active) but not for 5 and 6.

Both hydralazine and the new compound, 4, relaxed NA- and K*--induced contractions of rat aorta dose dependently and nonspecifically. These in vitro results correlate well with the in vivo findings and, although the active dose of hydralazine in aorta is considerably greater than the concentration reached in plasma21 (possibly because of biotransformation to active metabolites),22 they suggest that the hypotensive and antihypertensive action of this drug may be due basically to its effects on vascular smooth muscle cells.

High K* concentrations cause strong contraction of rat aorta tissue by depolarizing smooth muscle cells and so increasing the influx of calcium through L voltage-dependent channels.34 Also, activation of a,-adrenergic receptors in rat aorta by NA induces a two-phase process in which an initial, fast, transient contraction caused by the inositol 1,4,5-trisphosphate-mediated release of calcium from intracellular stores is followed by a slow, sustained contraction caused by Ca** influx through receptor-operated Ca** channels.33 The results presented herein show that hydralazine and 4 relax both K*-- and NA-induced contractions with equal effectiveness. These results suggest that their primary action occurs within the cell (as has been reported for the action of hydralazine in human arteries28), although there may also be a secondary, nonelective action on the cell membrane that would consist of blocking calcium influx through voltage-dependent and receptor-operated channels in agreement with previous studies of the action of hydralazine in rat tail artery.49,50

Hydralazine and 4 reduced neither basal uptake of **Ca nor uptake induced by NA and K*. Contrary to the results obtained by McLean et al.35 and Weiss et al.35 for hydralazine using rabbit aorta. These results suggest that hydralazine and 4 do not, after all, block transmembrane calcium movements through leak, voltage-dependent, and receptor-operated calcium channels in rat aorta.

In the absence of external Ca**, addition of NA is known to induce a fast, transient contraction attributed to release of stored Ca**; followed by a smaller, slow, sustained contraction whose mechanism is not clear although it has been shown that diacylglycerol derived from phosphatidylinositol breakdown activates protein kinase G and that activation of protein kinase C induces a sustained contraction in the presence of a low concentration of Ca**.30 In this work, hydralazine and 4 inhibited both phases of the contraction elicited by NA in calcium-free solution, showing that it acts intracellularly (as reported by Lipe and Moulds36 for the action of hydralazine in human arteries and veins). This action may consist of inhibition of inositol 1,4,5-trisphosphate-induced release of intracellular calcium, of direct action on the contractile apparatus (as reported by Jacoby37 for the action of hydralazine in bovine carotid arteries), or of activation of intracellular storage of Ca**. It is unlikely that this intracellular action is due to an increased rate of calcium loss (directly or indirectly via an increase in cyclic nucleotides), as hydralazine does not stimulate calcium-dependent ATPase in rat aorta.32

Cromakalim and other potassium channel openers relax contractions induced in various vascular preparations by low (<25 mM) but not high (>30 mM) K+ concentrations. This is the behavior expected of agents that only open K* channels.33 However, hydralazine and 4 relax contractions induced by high potassium concentrations (60 mM). Thus, hydralazine and 4 do not exert their vasorelaxant effects in rat aorta by opening membrane K* channels.

In this study we have looked at the mechanism of the vasodilatory action of hydralazine in rat aorta without endothelium. The effects described do not, therefore, involve the action of drugs on the vascular endothelium with consequent effects on the release of endothelial factors. Likewise, previous studies in our laboratory have indicated that hydralazine has identical effects on rat aorta with or without endothelium. These results, ruling out any effect of hydralazine on the release of endothelial factors, are in agreement with those of Bullock et al.34 in rat aorta, but contradict those of Spokes et al.35 in rabbit aorta. It is also of interest that some of the mechanisms susceptible to modulation by hydralazine and 4 are, however, altered in SHR smooth muscle.38-40 For this reason, and considering that our preliminary results indicate that SHR aorta rings have higher sensitivity to the vasodilatory effects of hydralazine, it may be of interest to study the vasodilatory action of hydralazine in aorta of genetically hypertensive rats.

References and Notes

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