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Changes in vascular reactivity in experimental hypertensive animals following treatment with indapamide

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Indapamide at doses of 8–16 mg kg^{-1} day^{-1}, orally, lowered arterial blood pressure (9–26 mm Hg) in conscious renal hypertensive cats during a two week treatment period. The antihypertensive effect was sustained for 5–7 h after dosing and was not accompanied by reflex tachycardia. Antihypertensive responses to injection of clonidine (20 μg, i.c.v.) were significantly enhanced one week after the completion of indapamide treatment but had returned to normal two weeks later. In DOCA/saline hypertensive rats, administration of indapamide 10 mg kg^{-1} day^{-1}, orally, or hydrochlorothiazide, 5 mg kg^{-1} day^{-1}, intraperitoneally, for 10 days produced similar falls in blood pressure (40–45 mm Hg) as measured by an indirect method. Pressor responses to intravenous noradrenaline or tyramine or electrical stimulation of the sympathetic outflow in the pithed rat preparation were much reduced by pretreatment with indapamide (10 mg kg^{-1}, orally) for 10 days. However, cardiovascular reactivity was unaffected by hydrochlorothiazide pretreatment (5 mg kg^{-1} day^{-1}, i.p.). Isolated perfused mesenteric artery preparations from indapamide-treated rats showed no changes in reactivity to noradrenaline, 5-hydroxytryptamine or adenosine-5′-triphosphate from those of control DOCA/saline hypertensive rats. Isolated portal veins from rats pretreated with indapamide showed contractile responses to noradrenaline similar to those of control animals although the frequency of spontaneous contractions was reduced in the former group. The results support a vascular site of action for indapamide and suggest a mode of action different from that of hydrochlorothiazide.

Indapamide has been shown to be an effective antihypertensive agent in man (Brun & Grunwald, 1976; Royer, 1976) and experimental hypertensive animals (Kyncl, Oheim & others, 1975; Finch & Hicks, 1976). The mode of action is not clearly understood although it decreases contractions of isolated vascular tissues to various agents (Kyncl & others, 1975) and the antihypertensive effect in man is accompanied by a reduction in peripheral resistance (Canicave & Lesbre, 1976).

We now report that indapamide reduces vascular reactivity to various pressor stimuli in DOCA/saline hypertensive rats. This is of interest because it is known that an increased reactivity occurs in hypertensive patients and also in experimental hypertensive animals (Folkow, 1971, 1976; Bohr & Berecek, 1976; Finch, 1971, 1974a, 1975). Various diuretic agents have already been reported to reduce pressor responses in models of experimental hypertension in the rat (Nicholas, 1971). We have therefore compared the actions of indapamide with those of hydrochlorothiazide at doses which produce similar antihypertensive effects. In addition, the antihypertensive action of indapamide has been examined in the conscious renal hypertensive cat.

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MATERIALS AND METHODS

DOCA/saline hypertensive rats

In rats weighing 120–140 g, the left kidney was removed under ether anaesthesia, and two slow-release tablets each containing 25 mg deoxycorticosterone acetate (DOCA) were implanted subcutaneously. The animals were given 0·9% w/v saline to drink for the next four weeks. After a further 4 weeks, the systolic blood pressure was measured indirectly in conscious animals using a tail cuff method (Finch, 1971). Groups of hypertensive rats (n = 5–9) were treated orally with indapamide, 10 mg kg^{-1} (suspended in carboxymethylcellulose), the vehicle alone or hydrochlorothiazide 5 mg kg^{-1} (i.p.) for 10 days. Blood pressure was measured before dosing began and the day after treatment ended (day 11). The following experiments on vascular reactivity were performed on day 11.

Pithed rat preparation

Under anaesthesia (sodium pentobarbitone 60 mg kg^{-1}, i.p.) rats were pithed through the right orbit with a steel rod and immediately artificially respired. Blood pressure was recorded from the right carotid artery using a Bell and Howell pressure transducer

connected to a Devices recorder. Drugs were injected into the left jugular vein. In some experiments the complete sympathetic outflow was stimulated according to Gillespie & Muir (1967). In these preparations, the animals were treated with (+)-tubocurarine (1 mg kg^{-1}, i.v.) and atropine (0·5 mg kg^{-1}, i.v.) before commencement of stimulation. Submaximal stimulation was applied to the pithing rod from a Grass S 9 stimulator with an intensity of 25 V, 1 m s and 1–20 Hz for periods of 10 s. The indifferent electrode was placed under the skin. Pressor responses to intravenous administration of noradrenaline or tyramine were also studied.

Isolated perfused mesentery preparation

Vascular reactivity to noradrenaline, 5-HT and ATP were determined in the isolated Krebs-perfused meserteric artery preparation according to Haeusler & Finch (1972), preparations from treated and control animals being compared simultaneously using a double organ bath.

Isolated portal vein preparation

Rats were killed by a blow on the neck and the portal veins were dissected out and each mounted under a tension of 1 g in a 20 ml organ bath containing oxygenated Krebs solution at 37°. Isometric tension was recorded using a Devices strain gauge and recorder. The preparation was allowed 45 min to equilibrate then a dose-response curve to noradrenaline was determined by sequential administration of increasing concentrations of the amine. The exposure periods were of 3 min duration with intervals of 15 min which allowed complete recovery of the normal spontaneous activity.

Conscious renal hypertensive cats

Five cats (2–3·5 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.p.) and were made hypertensive by wrapping one kidney with Cellophane and by contralateral nephrectomy. At a separate operation a cannula was passed down the right carotid artery and the tip located in the thoractic aorta. The cannula was exteriorized at the back of the neck and was attached to a one-way valve secured to the skin (Finch, 1974b). At the same operation, an indwelling intracerebroventricular (i.c.v.) cannula was implanted to allow administration of drugs into the lateral brain ventricles (Finch, 1974b). Hypertension developed over 3–6 weeks following the initial operation. For blood pressure recordings, the cats were placed in cages and 2 h allowed for basal blood pressure to stabilize. Recordings of mean blood pressure were made throughout each day on 5 days a week for 4 consecutive weeks. During the first week no treatment was given; in the second week empty gelatin capsules were administered orally. During weeks 3 and 4 respectively the cats received indapamide 20 and 40 mg daily in gelatin capsules. Three weeks before treatment began and 1 and 3 weeks after completion of treatment with indapamide, the antihypertensive response to clonidine (20 μg, i.c.v.) was determined in each cat.

Drugs used. Indapamide (1520 SE) 1-(4-chloro-3-sulfamyl benzamido)-2-methyl-indoline (Servier Laboratories Ltd, Greenford), noradrenaline acid tartrate (Hoechst), (+)-tubocurarine (Wellcome Labs), atropine sulphate (Northern pharmaceuticals); hydrochlorothiazide (Merck, Sharpe & Dohme), tyramine HCl (BDH); 5-hydroxytryptamine creatinine sulphate (Koch-light); adenosine-5′-triphosphate (ATP, Koch-light); clonidine hydrochloride (Boehringher Ingelheim). Concentrations of noradrenaline and 5-HT are expressed in terms of the base.

Statistical analysis. All results are expressed as mean ± standard error and n is the number of individual results for each group. For evaluation of statistical significance, Student's *t*-test was used.

RESULTS

Antihypertensive effect of indapamide in the conscious renal hypertensive cat

Daily oral administration of 20 mg (6–8 mg kg^{-1}) produced a slight antihypertensive effect (9 mm Hg). When the dose was increased to 40 mg (12–16 mg kg^{-1}) a more pronounced effect was observed (22–26 mm Hg) but this was not accompanied by reflex tachycardia (Fig. 1). The antihypertensive effect was sustained for 5–7 h. Three weeks before the first dose of indapamide the mean antihypertensive response to clonidine (20 μg, i.c.v.) was 49 ± 3 mg Hg. However, one week after treatment had stopped the response was significantly increased to 70 ± 3 mm Hg ($P < 0{\cdot}01$) but had returned to normal (45 ± 2 mm Hg) after a further two weeks.

Effect of indapamide and hydrochlorothiazide on the blood pressure of the conscious DOCA/saline hypertensive rat

Indapamide (10 mg kg^{-1}) administered orally for 10

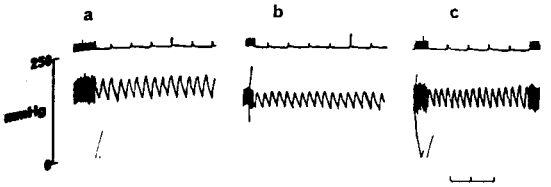

FIG. 1. *Effect of indapamide 12·5 mg kg^{-1}, orally, on the resting blood pressure of a conscious renal hypertensive cat (♀, 3·2 kg). Blood pressure was recorded from a catheter chronically implanted in the thoracic aorta. The changes in heart rate represent spontaneous variations in cardiac rate. There were no significant changes indicative of drug-induced tachycardia. a—Control, b—2 h, c—5 h. Horizontal scale represents time (s).*

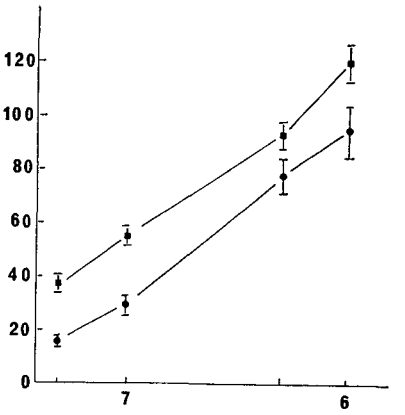

FIG. 2. Effect of indapamide on pressor responses to noradrenaline in pithed DOCA/saline hypertensive rats. Controls (■); rats pretreated with indapamide (10 mg kg^{-1} day^{-1}, orally) for 10 days (●); n = 6 for both groups. Ordinate: Rise in mean blood pressure (mm Hg). Abscissa: Noradrenaline (−Log g kg^{-1}).

days produced a marked fall in blood pressure of 45 mm Hg measured using the indirect tail/cuff method (Table 1). Treatment with hydrochlorothiazide (5 mg kg^{-1}, i.p.) for the same period produced a similar antihypertensive effect but animals treated with the vehicle alone showed no significant change in resting blood pressure (Table 1). In preliminary studies, hydrochlorothiazide administered orally at 5 or 10 mg kg^{-1} did not produce any significant changes in blood pressure although at high doses a marked hypokalaemia was observed.

Effect of indapamide and hydrochlorothiazide on vascular reactivity in the pithed rat preparation

Pretreatment with indapamide (10 mg kg^{-1}, orally) markedly decreased the vascoconstrictor responses to noradrenaline ($P < 0.02$) in the pithed rat preparation (Fig. 2). Pretreatment with hydrochlorothiazide (5 mg kg^{-1}, i.p.) lowered blood pressure to the same extent as did indapamide but did not significantly change vascular reactivity to noradrenaline. In the same preparations, intravenous administration of tyramine (10 and 50 μg kg^{-1}) increased mean blood pressure in the control group by 27 ± 5 and 69 ± 7 mm Hg respectively. In the indapamide-pretreated animals the corresponding increases were only 14 ± 2 and 38 ± 4 mm Hg and the differences between these and control responses were significant ($P < 0.05$). Hydrochlorothiazide pretreatment did not significantly change pressor responses to tyramine. In the Gillespie and Muir preparation, stimulation of the entire sympathetic outflow produced frequency dependent pressor responses which were significantly decreased ($P < 0.05$) up to 50% in animals which had received indapamide (10 mg kg^{-1}, orally) for 10 days (Fig. 3). However, pretreatment with hydrochlorothiazide (5 mg kg^{-1}) did not significantly affect pressor responses to electrical stimulation of the sympathetic outflow.

Table 1. *Effect of administration of indapamide and hydrochlorothiazide for 10 days on the mean systolic blood pressure of conscious DOCA/saline hypertensive rats as measured using an indirect tail/cuff method.*

Treatment	Systolic blood pressure (mm Hg) Before dosing began	After last dose
Indapamide 10 mg kg^{-1} day^{-1}, orally (n = 5 for both groups)	172 ± 5	127 ± 5*
Hydrochlorothiazide 5 mg kg^{-1} day^{-1}, i.p. (n = 9)	170 ± 7	130 ± 5*
Vehicle control orally (n = 8)	172 ± 6	166 ± 7

* $P < 0.001$.

Effect of indapamide on vascular reactivity in the perfused mesentery preparation and isolated portal vein preparation

The vasoconstrictor responses to noradrenaline, ATP and 5-HT in the perfused mesentery preparations were not reduced by pretreatment of animals with indapamide (10 mg kg^{-1}, orally). The dose-response curves for noradrenaline in control and indapamide-pretreated animals are shown in Fig. 4. Isolated portal vein preparations also showed no

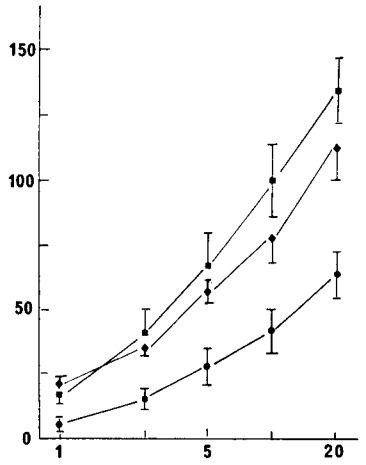

FIG. 3. Effect of indapamide and hydrochlorothiazide on pressor responses to electrical stimulation of the entire sympathetic outflow in pithed DOCA/saline hypertensive rats. Controls (◆); indapamide pretreatment was (10 mg kg^{-1} day^{-1}, orally) for 10 days (●); hydrochlorothiazide pretreatment (5 mg kg^{-1} day^{-1}, i.p.) was for 10 days (■); n = 6 for both groups. Ordinate: Rise in mean blood pressure (mm Hg), Abscissa: Electrical stimulation (Hz).

change in sensitivity to noradrenaline following indapamide administration. However, the spontaneous rhythmic contractions of the portal veins were reduced in frequency by up to 30% in preparations from indapamide pretreated rats.

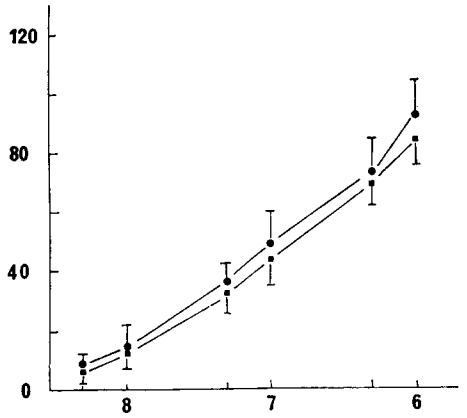

FIG. 4. Dose-response curves for the increase in perfusion pressure produced by noradrenaline in isolated perfused mesenteric artery preparations from control (●) and indapamide pretreated (■) DOCA/saline hypertensive rats. Indapamide pretreatment (10 mg kg^{-1} day^{-1}, orally) was for 10 days before the experiment. n = 8 for both groups. Ordinate: Rise in mean blood pressure (mm Hg). Abscissa: −Log g noradrenaline.

DISCUSSION

Daily oral administration of indapamide to conscious renal hypertensive cats produced an appreciable fall in arterial blood pressure. This antihypertensive action was well maintained throughout the two week treatment period and was not accompanied by reflex tachycardia. These observations confirm the previous work in the renal hypertensive dog (Kyncl & others, 1975). Antihypertensive responses to clonidine (i.c.v.) were significantly elevated one week after indapamide treatment had ended but had returned to normal two weeks later. These results suggest that indapamide exerts a long-lasting effect on cardiovascular control in hypertension. This is supported by the continuation of antihypertensive effects in man for 6–8 weeks after treatment ends (Hamilton & Kelly, 1977). The long duration of action of indapamide would appear to be related to a comparatively slow elimination of the drug from the plasma (Campbell, Taylor & Moore, 1976).

Indapamide showed marked antihypertensive activity after repeated oral administration in DOCA/saline hypotensive rats as was previously reported for these and spontaneously hypertensive rats (Kyncl & others, 1975). Hydrochlorothiazide was ineffective by mouth but lowered blood pressure after parenteral administration as shown by Stanton & White (1965). Rats pretreated for 10 days with indapamide showed a large decrease in vascular reactivity in the pithed preparation. In contrast to this, however, pretreatment with hydrochlorothiazide failed to modify pressor responses to noradrenaline or electrical stimulation of the sympathetic outflow despite achieving an antihypertensive effect similar to that of indapamide. This does not confirm the previous observation of a reduction in pressor responses in DOCA/saline hypertensive rats (Nicholas, 1971). However, in the latter study higher doses were used and the animals were examined shortly after dosing. Other *in vivo* experiments on vascular reactivity following thiazide treatment have given either negative (Jandhyala, Cavero & Buckley, 1972), positive (Zsoter, Hart & Radde, 1970) or equivocal results (Aoki & Brody, 1969). However, diazoxide administration impairs pressor responses to electrical stimulation of the sympathetic outflow and to various pressor agents in pithed renal hypertensive rats (Hamilton & Robson, 1975). The present findings with indapamide therefore show a qualitative difference to those obtained with thiazide diuretics in experiments *in vivo*.

The vasoconstrictor responses to noradrenaline, 5-HT and ATP in the isolated perfused mesentery preparation were unaffected by pretreatment of the rats with indapamide for 10 days. A similar lack of effect has been observed with hydrochlorothiazide pretreatment in the dog (Clarke, Ertel & others, 1972) and with drug combinations of hydrochlorothiazide / reserpine / hydrallazine in DOCA/saline (Finch, 1974a; 1975) and spontaneously hypertensive rats (Hamilton, 1975). There are major differences between the pithed rat and perfused mesentery preparations which may possibly explain the absence of effect of indapamide in the latter case. Firstly the mesentery preparation contains only arteries and arterioles and lacks the resistance vessels present in the intact vascular beds of the pithed animal (Finch, 1974a). Secondly, the former preparation utilized drug-free physiological saline as the perfusate. This is in contrast to the situation which prevailed in the pithed animals since a comparatively high proportion of the dose given is located in the blood (Campbell & others (1976) and after 10 days treatment drug and metabolite concentrations would have been in steady state. Moreover, perfusion of hindquarter preparations of DOCA/saline hypertensive rats with Krebs solution containing indapamide 6×10^{-5} M does reduce vasoconstrictor responses to noradrenaline (Moore, unpublished).

Pretreatment with indapamide also failed to impair contractile responses of isolated portal veins to noradrenaline. However, the frequency of spontaneous contractions was reduced in preparations from indapamide-pretreated rats. Incubation with frusemide has also been reported to decrease the amplitude of spontaneous contractions of this preparation (Blair-West, McKinley & McKenzie, 1972). Furthermore the absence of indapamide in the incubation medium was probably crucial since in the presence of indapamide, 3×10^{-5} to 3×10^{-4} M contractions of this preparation to noradrenaline are reduced (Parratt, private communication).

In conclusion, indapamide exerts an antihypertensive, action in conscious renal hypertensive cats and DOCA/saline hypertensive rats which is not accompanied by tachycardia. Vascular reactivity in the pithed rat preparation is much reduced by pretreatment with indapamide for 10 days but is unaffected by hydrochlorothiazide. The results suggest a direct action of indapamide on the vascular bed and a mode of action different from hydrochlorothiazide.

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