Antihypertensive and other cardiovascular effects of 2-(3-pyridyl)- and 2-(4-pyridyl)-4-trifluoromethylimidazoles

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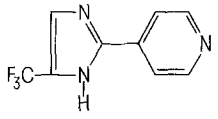
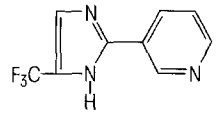
Summary. 2 new 4-trifluoromethylimidazole derivatives were found which lowered mean arterial pressure in renal and spontaneously hypertensive (SH) rats by the oral route. In SH rats, compounds A and B were 0.1 and 0.3 times, respectively, as potent as hydralazine. No tolerance development was observed in SH rats with either compound over a 1-week period. In anesthetized dogs, both compounds lowered arterial pressure and peripheral vascular resistance but increased cardiac output. By intraarterial administration, both compounds increased femoral arterial blood flow. These findings represent discovery of a new class of vasodilator drugs.

A search for novel antihypertensive drugs led us to the discovery of antihypertensive activity in a series of 4-trifluoromethylimidazoles. Some aspects of structure-activity relationship in this series of compounds were previously reported[3,4]. This publication deals with the antihypertensive and other cardiovascular effects of 2 of the more potent 4-trifluoromethylimidazoles. The chemical structures of 2-(4-pyridyl)-4-trifluoromethylimidazole (compound A) and 2-(3-pyridyl)-4-trifluoromethylimidazole (compound B; MK-534) are shown below:



Compound A



Compound B (MK-534)

The antihypertensive activity of compounds A and B was studied in both renal hypertensive and spontaneously hypertensive rats. Renal hypertension was produced in male Camm Sprague-Dawley rats of 120 to 190 g b.wt. The animals were made hypertensive by right nephrectomy and ligation of the caudal left renal artery branch. Hypertension gradually developed over a period of 2 to 4 months and the animals were used for evaluation of drugs when they reached a b.wt of 400 g. Male spontaneously hypertensive (SH) rats of the Wistar-Okamoto strain were obtained from Purina Farms (Vincentown, NJ) and allowed to reach a b.wt of 300 to 350 g. Arterial pressure was recorded continuously in conscious animals on a Honeywell Model 906C recorder or on a Gilson M-5 polygraph by a direct technique involving cannulation of the caudal artery as described by Watson and Ludden[3].

The effects of both drugs on cardiac output were studied in mongrel dogs of either sex and 7 to 14 kg b.wt anesthetized with sodium vinbarbital, 60 mg/kg i.v. Cardiac output was recorded by a dye dilution technique according to the method of Hamilton et al.[6]. Indocyanine green dye (Cardio-Green®) was rapidly injected into the jugular vein. Dye concentration in arterial blood was recorded with Gilford densitometer Model 103 and Constant Flow System Model 105-S. The area under the dye dilution curve was calculated with a Model 130 Hewlett Packard cardiac output computer. Aortic blood pressure, right atrial pressure, ECG (Lead II) and dye dilution curves were recorded with a Hewlett Packard Series 7700 8-channel recorder. Total peripheral vascular resistance (TPR) was calculated in dynes sec · cm^{-5} according to the formula:

$$TPR = \frac{(\text{Mean arterial pressure, mm Hg} - \text{right atrial pressure, mm Hg}) \times 1332}{\text{Cardiac output, ml/sec}}.$$

Both compounds were studied for their effect on papillary muscles isolated from the right ventricle of cat hearts according to the technique of Cattell and Gold[7]. The papillary muscles were placed in a 100-ml bath containing solution C of Thorp and Cobbin[8] and electrically stimulated with square wave shocks of 4 to 10 msec duration, 6 to 10 V and 1 per sec frequency from a Grass Model S4A stimulator. Isometric contractions were recorded through a Statham UC3 Universal transducing cell on a Honeywell Model 906 Visicorder or on a Hewlett Packard series 7700 recorder.

As shown in table 1, both compounds significantly lowered mean arterial pressure in renal hypertensive rats. The effect

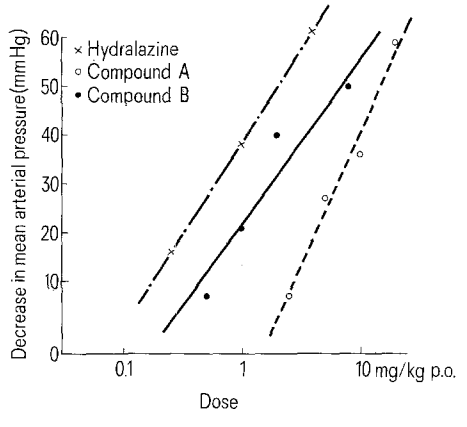


Fig. 1. Dose-response regression lines for antihypertensive effects of compounds A and B and hydralazine in spontaneously hypertensive rats. Maximal observed decreases in mean arterial pressure over 24 h after treatment are plotted against oral dose of the drugs. Average values for 4 to 12 animals per each dose of each compound.

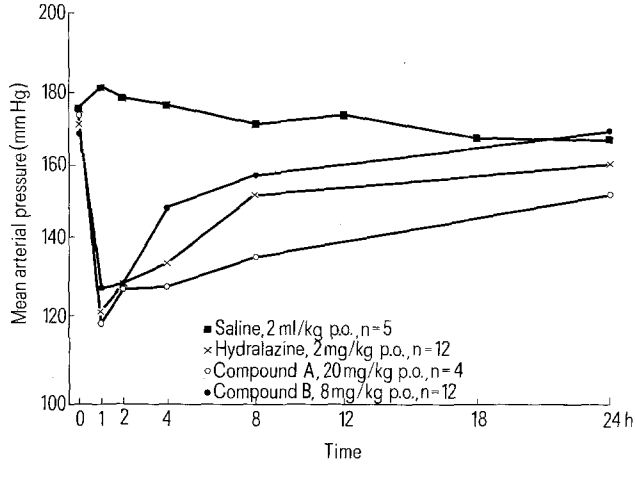


Fig. 2. Onset and duration of antihypertensive action of 4-trifluoromethylimidazoles (compounds A and B) and hydralazine in SH rats. N = number of animals per group. Mean arterial pressure is plotted against time in h. The compounds or saline were administered orally after 0 time readings.

Table 1. Antihypertensive activity of 4-trifluormethylimidazoles in renal hypertensive rats

Group	Drug	Dose (mg/kg p.o.)	Number of rats per group	Mean arterial pressure (mm Hg) at h after treatment 0	0.5	1	2	4	7	24
1	Compound A	2	2	195	181	187	184	175	179	174
2	Compound A	10	4	208	181*	185*	186*	184*	181*	181*
3	Compound B	1	4	175	165	167	168	169	164	176
4	Compound B	2	4	175	164	168	172	180	167	166
5	Compound B	8	4	161	117*	113*	109*	123*	146	159

*Significantly different from control values at 0 time.

Table 2. Hemodynamic effects of 4-trifluoromethylimidazoles in anesthetized dogs

Variable	Treatment compound*	At min after treatment 0	2	15	30
Mean arterial pressure (mm Hg)	A	137	114**	125**	124**
	B	108	59***	91***	99***
Cardiac output (l/min)	A	1.36	3.31**	1.49	1.46
	B	1.09	1.94**	1.48	1.13
Heart rate (beats/min)	A	165	166	160	160
	B	126	141	124	125
Right atrial pressure (mm Hg)	A	6	6	6	6
	B	7.3	7.2	6.7	6.8
Total peripheral vascular resistance, dynes sec · cm^{-5}	A	7788	3337**	6425**	6556
	B	7375	2250**	5005**	6644

Average values for 4 dogs per treatment group. *Compound A was given at 2.5 and compound B at 1 mg/kg i.v. **Significantly different from control value at 0 time, $p < 0.05$. ***Significantly different from control value at 0 time, $p < 0.01$.

Table 3. Effects of 4-trifluoromethylimidazoles on the contractile force of isolated cat heart papillary muscles

Group No.	Treatment	Concentration (µg/ml)	Number of experiments	Contractile force, mg at min after treatment 0	5	10	20	30
1	None		8	663	663	630	600	600
2	Compound A	10	4	400	400	475	550*	625*
3	Compound A After sotalol	10 10	2	450	450	450	450	450
4	Compound A Muscles from reserpinized animals	10	2	500	450	450	350	350
5	Compound B	10	4	500	488	463	425	488
6	Compound B	40	2	500	490	490	485	480

Average values for 2 to 8 preparations. *Significantly different from the control value at 0 time, $p < 0.05$.

was seen at 30 min after oral administration. The duration of action was dose-dependent; at 8 mg/kg p.o. the antihypertensive effect of compound B persisted for longer than 4 h while compound A, at 10 mg/kg p.o., was active for longer than 24 h.

In SH rats, compound B was active at 1 or more mg/kg p.o. and compound A at 5 or more mg/kg p.o. The dose-response regression lines of the 2 compounds and of hydralazine are shown in figure 1. Both 4-trifluoromethylimidazoles were less potent than hydralazine. In comparison with hydralazine (potency = 1.0), the relative potency of compound A was 0.1 with 95% confidence limits of 0.06 and 0.2; the relative potency of compound B was 0.3 with 95% confidence limits of 0.2 and 0.5.

The duration of antihypertensive action of compound A at 20 mg/kg p.o., of compound B at 8 mg/kg p.o. and of hydralazine at 2 mg/kg p.o. in SH rats exceeded 8 h. The maximal effect was observed at 1 h after treatment (figure 2). All 3 compounds produced a transient increase in heart rate which was more pronounced with compound B than with either compound A or hydralazine.

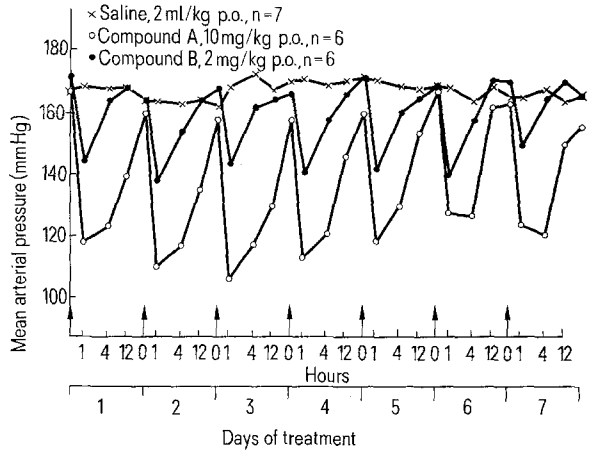


Fig. 3. Antihypertensive activity of compounds A and B by daily oral administration for 7 days. N = number of animals per treatment group. Mean arterial pressure is plotted against time in days and h. The compounes or saline were administered orally after 0 time readings.

In another series of experiments, compound A was administered at 10 and compound B at 2 mg/kg/day p.o. for 7 days each to 6 SH rats. The antihypertensive effect was observed on every treatment day; there was no evidence of tolerance development to either of the 2 compounds (figure 3).

By i.v. administration to anesthetized dogs, compound A at 2.5 mg/kg i.v. and compound B at 1 mg/kg i.v. lowered arterial pressure and peripheral vascular resistance. A pronounced increase in cardiac output was seen with both drugs at 2 min after treatment. Heart rate was slightly increased by compound B but not compound A. There was no significant change in right atrial pressure with either compound (table 2).

On isolated cat heart papillary muscles, compound A, 10 µg/ml, had slight positive inotropic activity. This effect was prevented by the β-adrenergic blocking agent, sotalol, 10 µg/ml 10 min prior to compound A or by pretreatment of cats with reserpine, 0.5 mg/kg i.p., 18 h prior to the test. Compound B at either 10 or 40 µg/ml had no effect on the contractile force (table 3). The positive inotropic effect of compound A was attributed to possible release of norepinephrine from cardiac storage sites.

Other exploratory experiments indicated that by intraarterial administration to anesthetized dogs, compounds A or B, 100 to 500 µg, transiently increased arterial blood flow. Neither of the 2 compounds had any ganglionic blocking, adrenergic neuron blocking or α-adrenoceptor blocking effects. In 2 cats with pithed spinal cord, compound A, 20 mg/kg i.v., lowered arterial pressure to an extent similar to that observed in normal cats. These experiments suggest that the hypotensive effect of compound A is likely to be independent of the autonomic nervous system and is possibly mediated by direct smooth muscle relaxant activity.

In preliminary toxicological studies, both compounds produced myocardial necrosis in dogs. In this respect, the 4-trifluormethylimidazoles were similar to directly-acting vasodilator drugs, e.g. hydralazine, diazoxide or minoxidil.

Our findings represent a discovery of a new class of vasodilator drugs which can conceivably be useful in the treatment of hypertension.

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Effects of chronic lithium administration on concanavalin A binding to plasma membranes from the corpus striatum of rat brain

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Summary. The effects of chronic in vivo lithium administration on mannose-containing components of plasma membranes from rat corpus striatum were examined by a ^{3}H-concanavalin A binding displacement method. No difference in Con A binding was observed between sodium or lithium-treated rats during a 1-month period.

Several diverse biological effects have been observed in association with the pharmacological action of the lithium ion (Li^+). These effects include depression of DNA polymerase activity[2], substitution for intracellular and extracellular cations[3], and altered metabolism of the catecholamines[4]. Furthermore, Li-induced changes in intracellular and extracellular function have initiated a number of theories of depression in association with the cell membrane[5]. Kline et al.[6] have reported observations by scanning electron microscopy on the choroid plexus of rats chronically treated with lithium which are compatible with possible alterations in surface glycoprotein hydration.

These potent neuropharmacologic actions of lithium have stimulated an examination of the effects of this ion on concanavalin A (Con A) binding in brain to examine possible structural changes in mannose-containing components of the cell membrane in a group of rats chronically treated with sodium or lithium.

Materials and methods. Male Sprague-Dawley rats weighing 160–200 g were maintained on Purina dry rat chow containing 0.21% (w/w) lithium or sodium in a 12-h light/dark cycle and were sacrificed at the end of 4 weeks. All animals treated attainer intracellular erythrocyte lithium levels between 0.4 and 0.8 meq/l.

After sacrifice by decapitation, whole brains were rapidly placed into chilled physiological saline. The corpora striata were dissected over ice, weighed and gently homogenized in a Kontes-Duall glass-teflon tissue grinder in 75 vol. of a modified Krebs Ringer buffer solution consisting of 11 mM NaCl, 0.5 mM KCl, 0.1 mM $MgSO_4$, 0.2 mM $CaCl_2$, 0.1 mM NaH_2PO_4, 1 mM ascorbic acid, 0.5 mM EDTA, 1 mM glucose, 5 mM $NaHCO_3$ and 0.1% (w/v) bovine serum albumin. The solution is oxygenated for 15 min, and pH adjusted to 7.4. All buffers and tissue homogenates were kept at 0–4 °C.

Binding of ^{3}H-labeled Con A (New England Nuclear, Boston, Mass. USA) to cell plasma membranes was measured by a method similar to that described by Cuatrecasas et al.[7] for fat cells. Tissue homogenate (containing 60 µg protein) was added to each assay tube containing 0.5 ml of the Krebs Ringer buffer, 2×10^4 cpm ^{3}H-Con A, and various amounts of unlabeled Con A (figure). Incubations were carried out in an ice bath for 90 min, after which each assay mixture was filtered over a Whatman glass fibre filter (GF/B) and the filter washed with an additional 10 ml of the ice-cold buffer. Total binding of the labeled lectin was measured as the excess over tubes containing no tissue. The molecular weight used for Con A was 100,000. Total