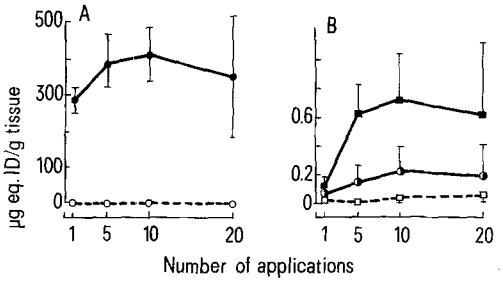

Concentration of (^{14}C)indomethacin (ID) in the skin (A) and muscle (B) of guinea-pigs by the topical application. ●—●, Skin at the applied site; ○----○, non applied skin; ■—■, shallow muscle under the applied site (ca. 3 mm in depth); ◑—◑, deep muscle (ca. 5 mm in depth); □----□, muscle under the non applied skin (ca. 3 mm in depth). Each symbol represents the mean value of 5 experiments. The vertical bars show SD.

the figure. The concentration of ID in the skin of the applied site increased with the repeated applications and reached a constant level of about 400 μg ID/g after 5 applications (figure 1A). The concentration of ID in the shallow part of the muscle under the applied site reached a constant level of about 0.7 μg ID/g after 5 applications, and that in the deep part of the muscle reached a level of 0.04–0.5 μg ID/g (about 0.2 μg ID/g in average) after 10 applications (figure 1B). The concentration of ID in the skin and muscle of nonapplied portion was negligible.

Discussion. It was reported that some drugs diffused into the muscle through the skin[1,2]. However, the p.c. penetration of drugs with repeated applications has not been well studied. The present experiments show that the topically applied ID penetrated through the skin, reached to the muscle as deep as 5 mm from the surface, and the concentration of ID in the muscle increased to a constant level after the several applications. Some drugs including anti-inflammatory agents are known to distribute preferentially in the inflamed tissue[3]. Therefore, the concentration of ID in the inflamed s.c. tissues attained by the topical application of ID may be higher than that in noninflamed tissues. The concentration of ID in the synovial fluid from arthritic patients after the oral administration of 50 mg of ID was reported to be 0.3–0.9 μg/ml[4]. The present study suggests that ID may possess anti-inflammatory activity in some s.c. soft tissues when applied to the skin as an ointment.

1 P. Pause, P. Zeiller and K.H. Sensh, Drug. Res. *24*, 1298 (1974).
2 H. Takahashi and K. Iwatsubo, Yakuzaigaku *23*, 49 (1963).
3 H.B. Hucker and E.A. Hoffman, J. Pharm. Sci. *60*, 1049 (1971).
4 H.W. Emori, G.D. Champion, R. Bluestone and H.E. Paulus, Ann. Rheum. Dis. *32*, 433 (1973).

Antihypertensive activity of some novel pyridinylidene arylurea derivatives in spontaneously hypertensive rats

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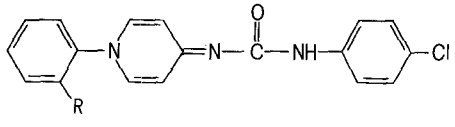
Summary. 3 novel pyridinylidene arylurea derivatives were found to lower arterial pressure in spontaneously hypertensive rats. Their relative oral potency ranged from 6 to 32 times that of guanethidine. The onset of antihypertensive action following their oral administration was less than 1 h and the duration of action ranged from 8 to over 24 h. The antihypertensive activity of the pyridinylidene arylureas was found to be associated with depletion of tissue catecholamines. Compound C depleted cardiac norepinephrine with little or no effect on total brain norepinephrine levels. It is suggested that compound C may have useful antihypertensive properties without CNS depressant activity.

In the search for antihypertensive drugs with novel chemical structures, we evaluated a series of antihypertensive pyridinylidene arylurea derivatives. A detailed structure activity study with this class of compounds will be published elsewhere[2]. The preliminary pharmacological studies with 3 compounds of this series are reported here. The chemical structures of the 3 selected compounds (A,B,C) are shown below:



Compounds	R
A	F
B	Cl
C	OCH_3

Spontaneously hypertensive (SH) rats of the Wistar Okamoto strain were purchased from Charles River/Lakeview Co. (Wilmington, MA). Arterial pressure was recorded in conscious male rats of 300–350 g b. wt by a direct technique involving cannulation of the caudal artery as described by Watson and Ludden[3]. Mean arterial pressure and heart rate values were printed at 0.5-h intervals through a data acquisition system (Data Graphics Corp., San Antonio, TX and Novatronics Co., Montgomeryville, PA) by means of ASR-33 teletype units. All drugs were administered in volumes of 2 ml/kg. Compounds A, B and C were suspended in 1% methylcellulose while guanethidine was dissolved in distilled water. The doses of all drugs were expressed in terms of base weight. Compound A was used as a fluorosulfonate salt, compound B as a free base, compound C as a hydrochloride salt and guanethidine as a sulfate salt. The calculations of the relative potency, its 95% confidence limits and regression lines were based on procedures described by Finney[4].

All 3 compounds were first tested in SH rats at 20 mg/kg i.p.; they lowered mean arterial pressure and reduced heart rate. At this dose, compound A was lethal in ½ and compound B in ¾ rats, while ½ rats which were treated with compound C survived. In further studies the compounds were administered p.o., each at 3 doses with 2–8 rats per dose. Their relative potency was calculated on the basis of maximal fall in arterial pressure over a 24-h period and compared with that of guanethidine which was previously evaluated under similar experimental conditions. The

Table 1. Relative antihypertensive potency of 3 pyridinylidene arylureas and guanethidine in SH rats by the oral route

Compound	Relative potency*	95% confidence limits**
Guanethidine	1	-
A	13	7-27
B	32	18-71
C	6	3-12

* 2-6 animals were used at each of 3 dose levels of each drug. The potency was calculated on the basis of maximal fall in mean arterial pressure over a 24-h period. ** Since the limits do not include the value of 1.0, they can be considered significant ($p < 0.05$).

results are summarized in table 1. If the potency of guanethidine was considered to equal 1, the relative potency of the three pyridinylidene arylureas was 13, 32 and 6 for compounds A, B and C respectively. All 3 compounds were significantly more potent than guanethidine and compound B was significantly more potent than compound C. The calculated dose-response regression lines for the three pyridinylidene arylureas are shown on figure 1. The statistical test for parallelism indicated that the regression lines were not significantly different from being parallel. The onset and duration of antihypertensive action of the pyridinylidene arylureas at 5 mg/kg p.o. and that of guanethidine at 20 mg/kg p.o. are compared in figure 2. The onset of antihypertensive action of all compounds was less than 1 h. The maximal effect was reached at 4 h for guanethidine and compounds A and C while the maximal effect of compound B was seen at 18 h after treatment. The maximal antihypertensive effect of compound B at 0.312 mg/kg p.o. was reached at 4-8 h while at 5 mg/kg p.o., the maximal effect was observed at 8-24 h. The effects of pyridinylidene arylureas at 5 mg/kg p.o. and guanethidine at 20 mg/kg p.o. on the heart rate of SH rats are shown in figure 3. Guanethidine at 20 mg/kg p.o. and compounds B and C at 5 mg/kg p.o. reduced heart rate while compound A had no significant cardiac slowing effects; at 20 mg/kg p.o. all 3 compounds reduced heart rate.

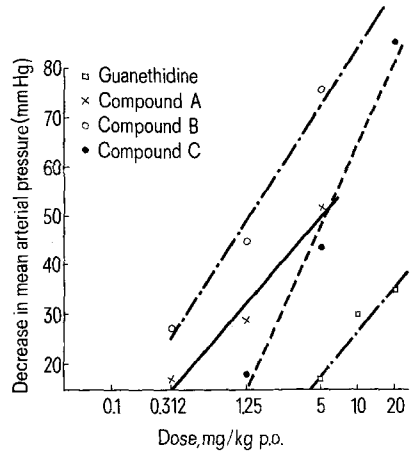


Fig. 1. Dose-response regression lines for antihypertensive effects of pyridinylidene arylurea derivatives (compounds A, B and C) and guanethidiine in SH rats. Maximal recorded decrease in mean arterial pressure is plotted against oral doses of the drugs. 2-8 animals were used at each dose level of each drug.

To evaluate the effects of the pyridinylidene arylureas on tissue catecholamine levels, male Sprague Dawley rats of 200-350 g b. wt were treated with the test compounds at 5 mg/kg in 2 ml/kg 1% methylcellulose p.o. 4-6 animals were used in each treatment group. A separate control group was used for each agent and analysis of control and experimental tissues were carried out at the same time. Tissue catecholamines were determined according to the differential fluorescence technique of Porter et al.[5]. The results are summarized on table 2. At the dose tested (5 mg/kg p.o.), compounds A and B significantly decreased brain norepinephrine and dopamine and heart norepinephrine levels whereas compound C decreased only cardiac norepinephrine. Other studies on the effects of the 3 compounds on catecholamine levels recently reported by Ulm et al.[6] confirmed these original findings.

To study in vitro inotropic activity of the pyridinylidene

Table 2. Effect of pyridinylidene arylureas at 5 mg/kg p.o. on tissue catecholamines in rats*

Compound	Brain Dopamine		Norepinephrine		Heart Norepinephrine	
	Control	Drug	Control	Drug	Control	Drug
A	6.79±0.98	1.24±0.39**	2.84±0.24	0.71±0.12**	4.91±0.24	1.06±0.41**
B	4.77±0.39	1.04±0.52**	4.14±0.35	1.30±0.41**	8.10±2.5	1.05±0.30**
C	5.59±0.41	5.49±0.25	4.35±0.43	4.35±0.30	6.38±1.06	1.32±0.43**

* 4 animals were used in each group; animals were sacrificed 6 h after treatment. ** Significantly different ($p < 0.001$) from corresponding control value as determined by Student's 2-tailed t-test.

Table 3. Effect of pyridinylidene arylureas on the contractile force of isolated cat heart papillary muscles

Drug	Concentration (µg/ml)	No. of experiments	Contractile force (mg±SE) Before treatment	30 min after drug	Maximal change in the contractile force during 30-min period±SE (%)
Saline 1 ml		12	433± 31	403±35	− 9± 5
Compound A	2.5	4	950± 50	1010±82	+ 6± 4
	10	5	932±142	672±82	−26± 8
	40	4	645± 42	305±59	−53± 8*
Compound B	2.5	6	463± 22	627±63	+30± 14*
	10	6	488± 29	453±29	−11± 3
	40	6	507± 48	253±46	−49± 8*
Compound C	2.5	6	407± 32	333±20	−17± 6
	10	6	487± 36	373±63	−21± 8
	40	6	460± 35	393±49	−14±10

* Significantly different from control experiments, $p < 0.05$.

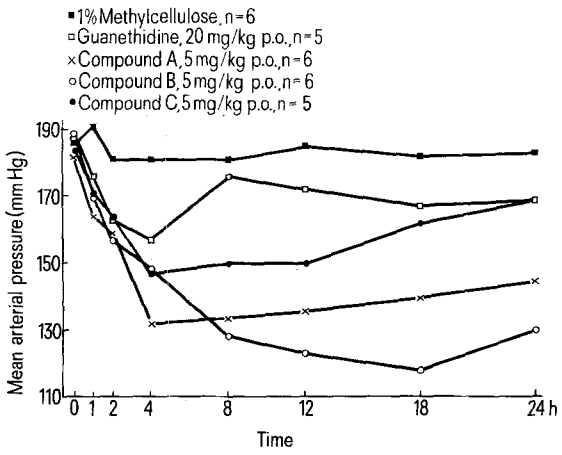


Fig. 2. Onset and duration of antihypertensive action of guanethidine and pyridinylidene arylureas in SH rats. N = number of animals per treatment group.

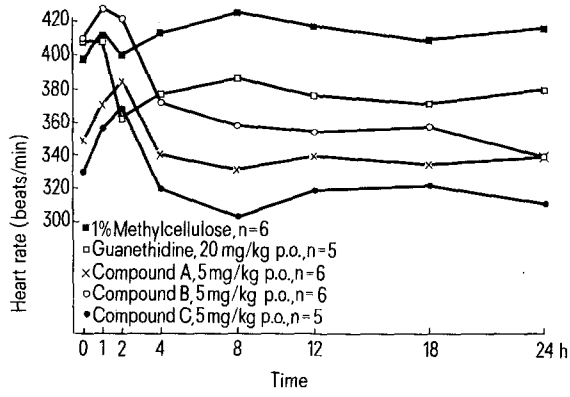


Fig. 3. Effects of guanethidine and pyridinylidene arylureas on heart rate in SH rats. Same experiments as in figure 2. N = number of animals per treatment group.

arylureas, papillary muscles from the right ventricle of cat hearts were isolated and electrically stimulated in accordance with the technique of Cattell and Gold[7]. None of the 3 compounds had significant myocardial depressant activity at 2.5 µg/ml although compounds A and B at 40 µg/ml reduced the isometrically recorded contractile force of papillary muscles (table 3). These findings suggest that the myocardial depressant effect is not likely to represent the major mechanism of the antihypertensive action of the pyridinylidene arylureas.

In preliminary behavioral studies in mice, all 3 compounds were tested at doses up to 150 mg/kg i.p. Compounds A and B but not C produced ataxia and ptosis and reduced exploratory behavior at doses below 50 mg/kg i.p. Compound C reduced exploratory behavior and produced ptosis only at doses exceeding 100 mg/kg i.p. Compound B was more toxic than compound A or C; its estimated 24-h LD_{50} in mice was 35.2 mg/kg i.p. whereas that of compound A or C was greater than 150 mg/kg i.p.

The findings in this study suggest that depletion of catecholamines represents a likely mechanism of the antihypertensive action of pyridinylidene arylureas. The relative inability of compound C to deplete brain catecholamines suggests that antihypertensive and central catecholamine depleting effects can be separated in this series of compounds and that compound C, while retaining antihypertensive activity, may be relatively free of CNS depressant properties of presently used catecholamine depleting agents, e.g. reserpine.

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2 M.H. Fisher, J.J. Fishinger, A.A. Patchett, W.V. Ruyle, C.T. Ludden, A. Scriabine, C.S. Sweet and E.H. Ulm, in preparation.
3 L.S. Watson and C.T. Ludden, in: New Antihypertensive Drugs, p. 87. Ed. A. Scriabine and C.S. Sweet. Spectrum Publications, Holliswood, NY, 1976.
4 D.J. Finney, in: Statistical Method in Biological Assay, 2nd ed, p. 99. Hafner Publishing Co., New York 1964.
5 C.C. Porter, J.A. Totaro and A. Burcin, J. Pharmac. exp. Ther. *150*, 17 (1965).
6 E.H. Ulm, C.T. Ludden, C.S. Sweet, D.H. Minsker, D. Haubrich, W.V. Ruyle, A. Scriabine and S.L. Gaul, Pharmacologist *20*, 188 (1978).
7 M. Cattell and H. Gold, J. Pharmac. exp. Ther. *62*, 116 (1938).

Depression of spontaneous and ionophore-induced neurotransmitter release by *Salmonella*[1]

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Summary. Exposure of frog neuromuscular junctions to heat-killed, lyophilized *Salmonella typhimurium* (SR 11) produces an early increase in spontaneous transmitter release followed by depression of release and blockade of the obligatory release usually induced by ionophore X537A.

Previous studies in our laboratory have shown that both crude and purified preparations of gram-negative bacteria depress neuromuscular transmission. Crude preparations of *Escherichia coli*, more commonly known as endotoxin, produce an increase, then a decrease in the frequency of miniature endplate potentials (MEPPs) recorded at the frog neuromuscular junction and block evoked endplate potentials (EPPs) by reducing quantal content[2]. The transient increase in MEPP frequency requires extracellular calcium. Purified preparations of the bacterial cell wall contain lipopolysaccharides (LPS) free of the protein contamination usually found in endotoxin. LPS similarly depresses spontaneous transmitter release but fails to produce the early, transient increase in MEPP frequency produced by endotoxin[3]. Sufficient exposure to LPS also apparently protects the presynaptic terminal from the usually destructive effects of the carboxylic cation ionophore X537A which, at untreated terminals, produces a transient rise in MEPP frequency to 100 times control levels followed by an abrupt abolition of both evoked and spontaneous transmitter release[4,5].

In general, the effect of LPS exposure seems to be one of