A study on the effects of some drugs on DOCA induced hypertension in the rat[1]

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Summary. Chronic treatment with various drugs reduced the increase in arterial blood pressure, the ventricular hypertrophy and the thickening of pulmonary arterial and arteriolar walls.

In order to make an experimental contribution to the use of drugs in the therapy of hypertension, some effects on the hypertension induced by DOCA have been evaluated in the rat.

We studied particularly the changes in arterial blood pressure, in the heart and in the pulmonary arteries and arterioles, in response to chronic treatment with antihypertensive drugs. The drugs used ranged from those with prevailing stimulating effects on central α-adrenoceptors (clonidine, flutonidine, guanabenz[3-5]), to those with β-adrenoceptor blocking properties (metoprolol[6]), or with pure α-adrenolytic properties (prazosin[7]), or with α-adrenolytic, anti-histamine and anti-serotonine properties (indoramine[8,9]), or with Ca^{++}-antagonistic properties (verapamil[10-13]) or finally with diuretic properties (hydrochlorothiazide[14], mefruside[15] and muzolimine[16,17]).

Materials and methods. The experiments were carried out on male albino rats(240-280 g Nossan strain). In conscious rats systolic arterial pressure was recorded indirectly from the tail by Roche BP Recorder[18]. The rats were treated with DOCA (5 mg/animal for 60 days, 3 times a week, i.m.) plus a drug as indicated in table 1, and drank saline solution (0.9%). All the doses of the drugs refer to their base. The following drugs have been used: clonidine [2-(2,6dichlorophenylamino)-2-imidazoline hydrochloride], guanabenz [2,8-dichlorobenzylidene aminoguanidine acetate], glutonidine [2-(5-fluoro-O-toluidine)-2-imidazoline hydrochloride, dl-metoprolol [1-isopropyl-amino-3[4-(2-methoxyethyl)-phenoxy]-2-propanol tartrate], prazosin [2-[4-(2-furoxyl)-piperazin-1yl]-4-amino-6,7-dimethoxyquinazoline hydrochloride], verapamil [α-isopropyl-α-[(N-methyl-N-homoveratryl)-γ-aminopropyl]-3,4-dimetoxyphenyl-acetonitrile hydrochloride], hydrochlorothiazide [6-chloro-3,4-dihydro-7-sulphamoyl-2H-benzo-1,2,4-thiadiazine], mefruside [N-(4'chloro-3'sulphamoyl-benzensulphonyl)-N-methyl-2-aminomethyl-2-methyl-tetrahydrophurano] and muzolimine [3-amino-1-(3,4-dichloro-methyl-benzyl)pyrazolin-5-one]. Statistical analysis of the results was carried out according to Burn's methods[19].

Results and discussion. The effect of various anti-hypertensive drugs on the hypertension induced by DOCA in the rat, on cardiac hypertrophy and on the caliber of the pulmonary arterial and arteriolar walls is reported in tables 1-4 respectively.

In rats, a 60-day treatment with clonidine (0.1 mg/kg/die p.o.), flutonidine (0.1 mg/kg/die p.o.), guanabenz (0.1 mg/kg/die p.o.), metoprolol (10 mg/kg/die i.p.), indoramine (10 mg/kg/die i.p.), prazosin (10 mg/kg/die p.o.), verapamil (5 mg/kg/die i.p.), hydrochlorothiazide (30 mg/kg/die p.o.), mefruside (10 mg/kg/die p.o.) and muzolimine (10 and 30 mg/kg/die p.o.) reduced the increase in

Table 1. Arterial blood pressure (mmHg) in DOCA hypertensive rats with or without drug treatment

Treatment (mg/kg/die)	Arterial blood pressure (mmHg ± SE) at the following days 30	60
Controls	134 ± 2.21	137 ± 3.26
DOCA[a]	192 ± 2.60	205 ± 7.63
Clonidine (0.1 p.o.) + DOCA[a]	158 ± 5.33[c]	158 ± 3.23[c]
Flutonidine (0.1 p.o.) + DOCA[a]	168 ± 7.02[c]	171 ± 5.32[b]
Guanabenz (0.1 p.o.) + DOCA[a]	150 ± 7.25[c]	175 ± 10.60[b]
metoprolol (10 i.p.) + DOCA[a]	168 ± 4.21[c]	159 ± 5.50[c]
Indoramine (10 i.p.) + DOCA[a]	164 ± 6.53[c]	170 ± 9.15[b]
Prazosin (10 p.o.) + DOCA[a]	156 ± 6.10[d]	145 ± 3.28[d]
Verapamil (5 i.p.) + DOCA[a]	170 ± 2.27[d]	157 ± 4.17[c]
Hydrochlorothiazide (30 p.o.) + DOCA[a]	180 ± 9.52 NS	178 ± 5.32[b]
Mefruside (10 p.o.) + DOCA[a]	161 ± 7.39[c]	152 ± 8.53[c]
Muzolimine (10 p.o.) + DOCA[a]	168 ± 6.05[c]	179 ± 4.42[b]
Muzolimine (30 p.o.) + DOCA[a]	130 ± 3.37[d]	176 ± 7.84[b]

Every value is the average of values from 10 rats.
[a] NaCl 0.9% ad libitum watered rats. DOCA (5 mg/i.m.) was administered 3 times a week. [b] $p < 0.02$ vs controls. [c] $p < 0.005$ vs controls. [d] $p < 0.001$ vs controls.

Table 2. Effects of drug-treatment on cardiac hypertrophy

Treatment (mg/kg/die)	Right ventricle (mm ± SE) A	B	C	A + B + C
Controls	0.47 ± 0.019	0.66 ± 0.019	0.72 ± 0.032	0.62 ± 0.031
DOCA[a]	0.88 ± 0.028	1.11 ± 0.02	1.58 ± 0.038	1.19 ± 0.204
Clonidine (0.1 p.o.) + DOCA[a]	0.53 ± 0.01[d]	0.74 ± 0.007[d]	0.83 ± 0.010[d]	0.70 ± 0.082[b]
Flutonidine (0.1 p.o.) + DOCA[a]	0.52 ± 0.02[d]	0.78 ± 0.030[d]	1.09 ± 0.030[d]	0.79 ± 0.141 NS
Guanabenz (0.1 p.o.) + DOCA[a]	0.56 ± 0.04[d]	0.87 ± 0.01[d]	1.27 ± 0.010[d]	0.90 ± 0.200 NS
Metoprolol (10 i.p.) + DOCA[a]	0.69 ± 0.01[d]	0.89 ± 0.010[d]	1.08 ± 0.011[d]	0.88 ± 0.110 NS
Indoramine (10 i.p.) + DOCA[a]	0.51 ± 0.02[d]	0.63 ± 0.010[d]	0.94 ± 0.019[d]	0.69 ± 0.121[c]
Prazosin (10 p.o.) + DOCA[a]	0.62 ± 0.02[d]	0.85 ± 0.021[d]	1.05 ± 0.013[d]	0.84 ± 0.120 NS
Verapamil (5 i.p.) + DOCA[a]	0.50 ± 0.03[d]	0.68 ± 0.010[d]	0.89 ± 0.020[d]	0.69 ± 0.112[c]
Hydrochlorothiazide (30 p.o.) + DOCA[a]	0.62 ± 0.01[d]	0.78 ± 0.010[d]	1.07 ± 0.010[d]	0.82 ± 0.130 NS
Mefruside (10 p.o.) + DOCA[a]	0.56 ± 0.01[d]	1.05 ± 0.01 NS	1.11 ± 0.007[d]	0.90 ± 0.171 NS
Muzolimine (10 p.o.) + DOCA[a]	0.79 ± 0.03[b]	0.61 ± 0.008[d]	1.11 ± 0.020[d]	0.83 ± 0.140 NS
Muzolimine (30 p.o.) + DOCA[a]	0.75 ± 0.03[b]	0.53 ± 0.01[d]	0.93 ± 0.010[d]	0.73 ± 0.112[b]

Every value is the average of values from 10 rats. A distance between epicardium and base of papillary muscles. B distance between epicardium and apex of smaller papillary muscle. C distance between epicardium and apex of larger papillary muscle. [a] NaCl 0.9% ad libitum watered rats. [b] $b < 0.02$ vs controls. [c] $p < 0.005$ vs controls. [d] $p < 0.001$ vs controls.

Table 3. Effects of drug-treatment on cardiac hypertrophy

Treatment (mg/kg/die)	Left ventricle (mm ± SE) A	B	C	A+B+C
Controls	1.39±0.035	2.20 ±0.032	2.51±0.039	2.03±0.127
DOCA[a]	1.92±0.029	2.93 ±0.030	3.96±0.042	2.93±0.540
Clonidine (0.1 p.o.)+DOCA[a]	1.08±0.008[d]	2.17 ±0.010[d]	3.21±0.078[d]	2.15±0.610 NS
Flutonidine (0.1 p.o.)+DOCA[a]	1.31±0.010[d]	1.09 ±0.102[d]	3.30±0.202[d]	2.23±0.570 NS
Guanabenz (0.1 p.o.)+DOCA[a]	1.64±0.040[c]	2.29 ±0.060[d]	3.23±0.072[d]	2.38±0.460 NS
Metoprolol (10 i.p.)+DOCA[a]	1.68±0.040[c]	2.52 ±0.030[d]	3.43±0.030[d]	2.54±0.500 NS
Indoramine (10 i.p.)+DOCA[a]	1.49±0.020[d]	2.37 ±0.042[d]	3.72±0.009[c]	3.53±0.640 NS
Prazosin (10 p.o.)+DOCA[a]	0.83±0.080[d]	2.04 ±0.022[d]	3.29±0.031[d]	2.05±0.710 NS
Verapamil (5 i.p.)+DOCA[a]	1.43±0.060[d]	2.42 ±0.021[d]	3.25±0.052[d]	2.36±0.520 NS
Hydroclorothiazide (30 p.o.)+DOCA[a]	1.22±0.030[d]	3.032±0.011[c]	3.58±0.060[d]	2.61±0.710 NS
Mefruside (10 p.o.)+DOCA[a]	1.08±0.030[d]	2.33 ±0.040[d]	3.44±0.151[d]	2.28±0.680 NS
Muzolimine (10 p.o.)+DOCA[a]	1.31±0.060[d]	2.14 ±0.020[d]	3.39±0.063[d]	2.28±0.600 NS
Muzolimine (30 p.o.)+DOCA[a]	1.25±0.060[d]	2.08 ±0.031[d]	3.26±0.060[d]	2.19±0.582 NS

Every value is the average of values from 10 rats. A Distance between epicardium and base of papillary muscles. B Distance between epicardium and apex of smaller papillary muscle. C Distance between epicardium and apex of larger papillary muscle. [a] NaCl 0.9% ad libitum watered rats. [b] $p<0.02$ vs controls. [c] $p<0.005$ vs controls. [d] $p<0.001$ vs controls.

Table 4. Effects of drug-treatment on the calibre of some pulmonary blood vessels in DOCA-hypertensive rat

Treatment (mg/kg/die)	Pulmonary artery (distance between 2 elastic borders of branchs with an about 100–200 μm calibre) (μm ± SE)	Pulmonary arteriolar vessel (distance between 2 elastic borders of arteriolar vessels with a 25 μm total diameter) (μm ± SE)
Controls	9.66±0.276	3.89±0.0280
DOCA[a]	15.81±0.470	12.47±0.310
Clonidine (0.1 p.o.)+DOCA[a]	11.98±0.321[d]	7.78±0.281[d]
Flutonidine (0.1 p.o.)+DOCA[a]	12.58±0.183[d]	8.90±0.370[d]
Guanabenz (0.1 p.o.)+DOCA[a]	12.94±0.400[a]	8.68±0.298[a]
Metoprolol (10 i.p.)+DOCA[a]	14.63±0.870[c]	11.70±0.294 NS
Indoramine (10 i.p.)+DOCA[a]	12.58±0.221[d]	7.87±0.163[d]
Prazosin (10 p.o.)+DOCA[a]	9.78±0.210[d]	6.26±0.045[d]
Verapamil (5 i.p.)+DOCA[a]	13.89±0.713[c]	7.84±0.184[d]
Hydroclorothiazide (30 p.o.)+DOCA[a]	12.22±0.391[d]	9.70±0.233[c]
Mefruside (10 p.o.)+DOCA[a]	14.30±0.540[c]	6.21±0.43[d]
Muzolimine (10 p.o.)+DOCA[a]	13.66±0.352[c]	5.92±0.072[d]
Muzolimine (30 p.o.)+DOCA[a]	11.78±0.141[d]	5.27±0.161[d]

Every value is the average of values from 10 male rats. [a] NaCl 0.9% ad libitum watered rats. [b] $p<0.02$ vs controls. [c] $p<0.005$ vs controls. [d] $p<0.001$ vs controls.

systolic arterial blood pressure, the ventricular hypertrophy, and the thickening of pulmonary arterial and arteriolar walls (tables 1–4) induced by DOCA.

The results suggest that an early beginning of antihypertensive treatment reduces the DOCA-induced increase in arterial blood pressure, the cardiac hypertrophy and the thickening of the walls of pulmonary arteries and arterioles. In previous studies in rats we have shown an antihypertensive effect of clonidine, flutonidine and guanabenz on DOCA-induced arterial hypertension[4]. Moreover it was also observed that metoprolol[6], similarly to other β-adrenolytic drugs, is able to diminish the following alterations induced by DOCA in the rat: arterial hypertension, reduction in pulse rate, increase in plasma renin concentration, platelet aggregation, S-GOT, S-GPT and S-LDH increase and thrombophilia.

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3 E. Marmo, A.P. Caputi, C. Vacca and R.K. Saini, Res. Commun. chem. Path. Pharmac. *6*, 391 (1973).
4 E. Marmo, A.P. Caputi, F. Rossi, E. Lampa, L. Giordano and G. Accarino, Agressologie *18*, 189 (1977).
5 E. Marmo, G. Nisticò, F. Rossi and D. Rotiroti, Res. Commun. chem. Path. Pharmac. *19*, 1 (1978).
6 A.P. Caputi, F. Rossi, E. Lampa, C. Vacca, L. Giordano and E. Marmo, Agressologie *19*, 325 (1978).
7 F. Lefèvre-Borg, A.G. Roach and I. Cavero, J. cardiovasc. Pharmac. *1*, 19 (1978).
8 E. Marmo, Rif. med. *94*, 145 (1979).
9 E. Marmo, F. Rossi, A.P. Caputi, E. Lampa, R. Petrarca, R. Ottavo, Clinica terap. *88*, 233 (1979).
10 A. Fleckenstein, H. Tritthart, B. Fleckenstein, A. Herbst and G. Grün, Pflügers Arch. *307*, R 25 (1969).
11 A. Fleckenstein, G. Grün and K.Y. Byon, Minerva med. Roma *66*, 1827 (1975).
12 E. Marmo, L. Coscia, F. Rossi, S. Chieppa, G. Brita and A.P. Caputi, Minerva med. Roma, *66*, 1771 (1975).
13 E. Marmo, Atti Giornata Cardiologica Catania, 31.3.1977. Pozzi, Roma 1977.
14 P. Preziosi, A.F. De Schaepdryver, E. Marmo and E. Miele, Archs int. pharmacodyn. Thér. *131*, 209 (1961).
15 K. Meng and G. Kroneberg, Arzneimittel-Forsch. *17*, 659 (1967).
16 E. Möller, H. Horstmann, K. Meng and D. Loew, Experientia *33*, 382 (1977).
17 F. Rossi, A.P. Caputi, P. Pedone, C. Vacca, D. Perna and E. Marmo, Riv. farmac. terap. *9*, 277 (1978).
18 M. Gerold and H. Tschirky, Arzneimittel-Forsch. *18*, 1285 (1968).
19 J.H. Burn, D.J. Finney and L.G. Goodwin, Biological standardization, 2nd ed. Oxford Med. Press, Oxford 1952.