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Influence of 1-(5-methyl-1-phenylpyrazol-4-yl)-3-[4-(*o*-tolyl)piperazin-1-yl]-propan-1-one hydrochloride (CIBA 1002-Go) on the stores of catecholamine in rat and cat tissues

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The effect of 1002-Go has been examined on the catecholamine stores in rat heart, brain, adrenals and cat heart. There is a significant reduction in the catecholamine content from all the tissues with doses ranging from 2·5–30 mg/kg. Guanethidine is slightly less potent as a depletor of catecholamine than 1002-Go in the rat. In cats, however, 1002-Go is much weaker than guanethidine or reserpine in depleting the catecholamine stores. There also seem to be differences among these antihypertensive agents in the duration of depletion and repletion of the catecholamine stores.

ANTIHYPERTENSIVE drugs differ in their mechanisms of action. Reserpine produces a profound and persistent alteration of the cellular mechanism for binding catecholamines (Green, 1962). α-Methyldopa depletes noradrenaline by a different mechanism; the drug is metabolized to α-methylnoradrenaline and this displaces noradrenaline from the binding sites (Andén, 1964). Guanethidine and bretylium block the postganglionic adrenergic transmission and possess a slight and transient ganglion-blocking activity. It has been suggested that they prevent the liberation of adrenergic transmitter from the nerve endings (Boura & Green, 1959; Maxwell, Mull & Plummer, 1959; Maxwell, Plummer & others, 1960). Guanethidine depletes catecholamine and this has been related to sympathetic blockade (Shepherd & Zimmerman, 1959; Cass, Kuntzman & Brodie, 1960). There is, however, evidence that the onset of sympathetic blockade does not parallel the rate of depletion of catecholamines (Cass & Spriggs, 1961; Sanan & Vogt, 1962).

The drug 1002-Go is a synthetic antihypertensive agent belonging to a group of phenyl piperazine Mannich products (Arya, Grewal & others, 1967). It lowers the blood pressure of renal hypertensive rats to normotensive level when given at 5–10 mg/kg twice daily (Grewal, Kaul & David, 1968). 1002-Go produces reversal of the effects of adrenaline at 0·25–0·5 mg/kg without any significant change in the noradrenaline pressor response. The compound blocks amphetamine and tyramine pressor responses in anaesthetized cats and dogs, an observation which suggests an interference with the release of catecholamines from the nerve endings (Burn & Rand, 1958). This block of amphetamine and tyramine pressor response is not related to the adrenolytic activity of the compound as 1002-Go has a very weak α-adrenergic blocking activity judged by diminution of noradrenaline response on blood pressure and aortic strips (Grewal & Kaul, unpublished observations). Since 1002-Go inhibits the pressor responses of amphetamine and tyramine, and many antihypertensive drugs are known to interfere with the release or normal distribution of the neurotransmitter at the sympathetic myoneural

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junction, it was interesting to see the effect of 1002-Go on the catecholamine stores in rat and cat tissues.

Experimental

METHODS

Male rats, from 140 to 160 g were used. Extraction of the tissues was by perchloric acid. The catecholamines were adsorbed on acid-washed alumina, pH 8·4 and eluted with 0·2N acetic acid as described by Crout, Creveling & Udenfriend (1961). For the assay of adrenals, the total extract was used. Noradrenaline from heart and brain was assayed on the blood pressure of a pithed rat using noradrenaline as a standard. The total catecholamines from adrenals were assayed on the spinal cat (blood pressure) using adrenaline as a standard.

Effect of 1002-*Go on the uptake of noradrenaline by heart.* The method used was that of Muscholl (1961) except that the rats were not pithed. Male rats from 140–160 g were treated with 5 mg/kg (i.p.) of 1002-Go. After 3 hr the rats were anaesthetized with urethane (15% 1·5 ml/100 g body weight). The jugular vein was cannulated and an infusion of noradrenaline (20 μg) was given at a constant rate for 20 min, using a motor-driven syringe. The total volume injected in any one experiment was not more than 2·5 ml. The rats were killed 5 min after the end of the infusion and noradrenaline from the heart was estimated on the blood pressure of the pithed rat. Control experiments were also made in which normal rats anaesthetized with urethane were given 20 μg of noradrenaline by infusion. These rats were killed 5 min after the end of the infusion and noradrenaline content of the heart was estimated.

In these uptake experiments infusion of noradrenaline was made in such a way that each rat was never anaesthetized for more than 1 hr and during this period urethane anaesthesia produces little change in the catecholamine content of the heart (Spriggs, 1965). The recoveries of added noradrenaline to tissue were 60–70%. Values reported are not corrected for the recoveries.

A solution of 1002-Go was prepared in warm polyethylene glycol. An equivalent volume of polyethylene glycol was given to control rats. Each series of experiments had its own controls. To minimize the likelihood of various factors influencing the results, a random order of treatment with the drug and the control solutions and subsequent procedures was used. All injections were made intraperitoneally.

Drugs used. (−)-Noradrenaline hydrogen (+)-tartrate, (−)-adrenaline hydrogen (+)-tartrate. Stock solutions of these two drugs were made in normal saline with 0·1N hydrochloric acid and further dilutions were made from the stock solutions. All concentrations and doses of noradrenaline refer to its salt, but the concentration of adrenaline refers to free base. Guanethidine was used as its sulphate.

Results

The effect of 1002-Go and guanethidine on the rat heart catecholamine content is shown in Table 1. It can be seen that a significant fall in rat

TABLE 1. *In Vivo* EFFECT OF 1002-GO AND GUANETHIDINE ON THE CATECHOLAMINE CONTENT OF THE RAT HEART AT DIFFERENT TIME INTERVALS AFTER TREATMENT

Treatment	Dose mg/kg	Time after treatment (hr)	Catecholamine conc. (μg/g ± s.e.†)
Controls ..	—	—	0·73 ± 0·03 (35)
		1	0·59 ± 0·05 (9)*
1002-Go ..	2·5	3	0·43 ± 0·05 (11)**
		6	0·54 ± 0·06 (7)**
		12	0·72 ± 0·05 (8)
Controls ..	—	—	0·67 ± 0·03 (35)
1002-Go ..	5	1	0·55 ± 0·03 (10)**
		3	0·18 ± 0·05 (7)***
		6	0·13 ± 0·02 (7)***
		12	0·67 ± 0·08 (8)
Controls ..	—	—	0·71 ± 0·04 (24)
Guanethidine ..	5	1	0·68 ± 0·058 (4)
		3	0·26 ± 0·06 (6)***
		6	0·30 ± 0·04 (8)***
		12	0·41 ± 0·05 (5)***

Figures in parentheses show the number of animals used.
† Values are not corrected for recoveries.
* $0·05 > P > 0·01$. ** $0·01 > P > 0·001$. *** $0·001 > P$.

heart catecholamine content occurred within 1 hr of treatment with 2·5 and 5 mg/kg of 1002-Go. At 3 hr both doses showed a highly significant effect, the onset of which was quite rapid with a return to normal levels within 12 hr. Guanethidine (5 mg/kg) seems to be slightly less potent as a catecholamine depletor than 1002-Go on the rat heart (Table 1).

A significant depletion of brain noradrenaline was effected by 10 mg/kg of 1002-Go 3 hr after the drug. The concentrations in μg/g were: control 0·40 ± 0·03; drug 0·10 ± 0·002 ($0·001 > P$). A dose of 30 mg/kg was necessary to produce a significant depletion of the adrenals. After 3 hr, the concentrations in μg/g were: control 969·9 ± 105; drug 514·4 ± 47 ($0·01 > P > 0·001$).

TABLE 2. *In Vivo* EFFECT OF 1002-GO, GUANETHIDINE AND RESERPINE ON THE CATECHOLAMINE CONTENT IN THE CAT HEART AT DIFFERENT TIME INTERVALS AFTER TREATMENT

Treatment	Dose mg/kg	Time after treatment (hr)	Catecholamine conc. (μg/g ± s.e.†)
Control ..	—	—	1·49 ± 0·11 (22)
1002-Go ..	5	3	1·38 ± 0·26 (4)
		6	1·60 ± 0·19 (8)
		12	1·26 ± 0·14 (8)
1002-Go ..	10	3	1·07 ± 0·05 (3)
		6	1·07 ± 0·11** (4)
		12	1·08 ± 0·11** (3)
1002-Go ..	20	3	0·85 ± 0·12*** (5)
		6	0·65 ± 0·04*** (3)
		12	0·80 ± 0·06*** (8)
Guanethidine	5	3	0·64 ± 0·15*** (3)
		6	0·57 ± 0·09*** (4)
		12	0·30 ± 0·07*** (6)
Reserpine ..	1	16	0·04 ± 0·008*** (4)

Figures in parentheses show the number of animals used.
† Values are not corrected for recoveries.
** $0·01 > P > 0·001$. *** $0·001 > P$.

The effect of 1002-Go, guanethidine and reserpine on the cat heart is shown in Table 2. There was a significant reduction in the catecholamine content at 3, 6 and 12 hr after 20 mg/kg of 1002-Go, and at 6 and 12 hr with the 10 mg/kg dose.

Reserpine (1 mg/kg) 16 hr after treatment caused a 97% depletion of catecholamine from the cat heart. Guanethidine (5 mg/kg) produced 61 to 81% reduction in the catecholamine content (Table 2). Thus guanethidine and reserpine would seem to be more potent than 1002-Go as catecholamine depletors on this preparation.

Table 3 shows the effect of 1002-Go on the uptake of infused noradrenaline by the rat heart. The means for the noradrenaline content in heart after the infusion are slightly lower in 1002-Go-treated rats than the controls, which suggests that 1002-Go interferes with the uptake of infused noradrenaline.

The effect of 1002-Go after long term treatment on the rat heart is shown in Table 4. Given for 10 days or given only once, 1002-Go (1 mg/kg) produced a similar reduction in the noradrenaline levels in the rat heart.

TABLE 3. EFFECT OF 1002-GO ON THE NORADRENALINE UPTAKE BY THE HEART AFTER AN INFUSION OF NORADRENALINE

Treatment	μg of noradrenaline infused in 20 min	Concentration of noradrenaline in μg/g
Control	—	0·59 ± 0·04 A
1002-Go, 5 mg/kg ..	—	0·18 ± 0·05 B
Control	20 μg	1·08 ± 0·11 C
1002-Go, 5 mg/kg ..	20 μg	0·48 ± 0·03 D

A highly significant difference was found between A and B, A and C, C and D but no significant difference between A and D.

TABLE 4. *In Vivo* EFFECT OF 1002-GO ON THE CATECHOLAMINE CONTENT OF THE RAT HEART

Treatment	Dose and time	Catecholamine conc. (μg/g ± s.e.*)
Controls	1 mg/day for 10 days	0·88 ± 0·03 (5)
1002-Go		0·58 ± 0·04*** (5)
Controls	1 mg	0·75 ± 0·08 (5)
1002-Go		0·47 ± 0·08 (5)†

Figures in parentheses show the number of animals used.

* Values are not corrected for recoveries.
† $0{\cdot}05 > P > 0{\cdot}01$.
*** $0{\cdot}001 > P$.

Discussion

The results show that 1002-Go, like many other antihypertensive drugs, depletes catecholamine in the rat and cat tissues. The depletion it

causes differs from reserpine and guanethidine in that its onset of action and the recovery of catecholamine is much faster than seen with reserpine and guanethidine (Cass & others, 1960; Orlans, Finger & Brodie, 1960). One hr after treatment, 1002-Go produced a significant fall in the catecholamine content of rat heart at 2·5 and 5 mg/kg. At 6 hr the effect was maximum and the normal levels were reached after 12 hr (Table 1). With guanethidine a highly significant effect was not observed until after 3 hr and the values were significantly lower than the controls even up to 12 hr, although the values tended to return to pretreatment levels after that time. These results are in agreement with those of Bogaert, De Schaepdryver & De Vleeschhouwer (1961) who found 53% depletion of catecholamine in the rat hearts 6 hr after 8 mg/kg of guanethidine and no effect after 24 hr. Cass & Spriggs (1961) have also shown 80–90% depletion of heart noradrenaline after guanethidine and normal levels were reached by 48 hr. The earlier recovery of catecholamine after guanethidine observed by Bogaert & others (1961) and by us may be because Cass & Spriggs (1961) used a higher dose of guanethidine (15 mg/kg) and secondly guanethidine was administered subcutaneously which might account for the longer duration of action.

The significant fall in brain noradrenaline at 10 mg/kg of 1002-Go and the significant lowering in the catecholamine content of the adrenals at 30 mg/kg are effects similar to, but less potent than, those of reserpine (Kirpekar, Goodlad & Lewis, 1958; Orlans & others, 1960). The failure of guanethidine to produce any significant change in the brain is not surprising because due to its low lipid solubility it is unlikely to cross the blood brain barrier readily (Cass & others, 1960).

On the cat heart a significant fall in the catecholamine content was observed only with the two higher doses of 1002-Go (10 and 20 mg/kg), but guanethidine and reserpine produced a marked fall in the catecholamine content even at low doses (5 and 1 mg/kg respectively).

Under our experimental conditions guanethidine seems to be less potent as a catecholamine depletor than 1002-Go in rats (Table 1), but both reserpine and guanethidine are more powerful than 1002-Go on the cat heart (Table 2). There also seems to be some difference among these three antihypertensive agents in the duration of depletion and repletion of catecholamine stores. 1002-Go has the shorter duration of action.

The rate of uptake of infused noradrenaline from the circulating blood is less in the presence of 1002-Go than in the controls; however, the block was not complete as is the case for reserpine (Muscholl, 1961).

The decrease in the catecholamine content seen after 1002-Go could be either due to the release of the amine or to the blocking of the synthesis. The depletion of catecholamines observed after long term treatment with 1002-Go, 1 mg/kg, is roughly the same as seen after a single dose (Table 4). This is probably because its effect does not last for a long time and repletion of catecholamine stores occurs rapidly. Our results would therefore suggest that depletion of catecholamine does not play a major role in the hypotensive effect of the compound.

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