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STRUCTURAL REQUIREMENTS FOR BRETYLIUM AND GUANETHIDINE-LIKE ACTIVITY IN A SERIES OF GUANIDINE DERIVATIVES

E. Costa, R. Kuntzman, G.L. Gessa and B.B. Brodie

Laboratory of Chemical Pharmacology

National Heart Institute

National Institutes of Health

Bethesda 14, Maryland

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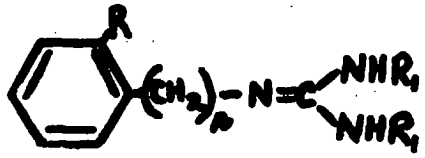
BRETYLIUM is unique among the sympatholytic drugs, since it is a quaternary ammonium compound which acts at nerve terminals to prevent the physiological release of the adrenergic transmitter.[1] This agent neither releases appreciable amounts of norepinephrine *in vivo*, nor antagonizes the effects of administered catecholamines. Guanethidine, another strongly basic drug, also blocks peripheral adrenergic mechanisms,[2] but like reserpine its action is associated with the depletion of peripheral stores of norepinephrine.[3,4] However, guanethidine and reserpine appear to act differently for experiments show that only the release of norepinephrine induced by guanethidine is blocked by bretylium-like drugs.[5] These results led to the speculation that guanethidine acts oppositely to bretylium, and depletes norepinephrine through persistent activation of the process by which the nerve impulse releases the amine.

Since the strongly basic groups in bretylium and guanethidine are separated from a ring by one or two carbon atoms respectively, we decided to study a series of compounds containing a benzene ring separated by one or two carbons from the highly basic guanidine group:



(R=H, Br, Cl; R_1=H or CH_3; n=number of carbon atoms)

Some of these guanidine derivatives were first studied by Boura et al.[6] who showed that they block the function of peripheral sympathetic neurons. Our results show that compounds having only a single carbon connection exert bretylium-like properties and prevent guanethidine from depleting heart norepinephrine; in contrast compounds with a two carbon chain exert guanethidine-like effects.

Methods

The guanidine derivatives (Table 1) were synthesized by The Wellcome Research Laboratories, Beckenham, England. Guanethidine-like activity was determined in Sprague Dawley rats by the reduction in heart norepinephrine, 7 hr after administration of drug (7.5 mg/kg i.v.).

Bretylium-like activity was measured by the ability of various doses of the drugs to prevent the loss of heart norepinephrine induced by guanethidine (5 mg/kg i.v.).

Heart norepinephrine was assayed by the spectrofluorimetric method of Shore and Olin.[7]

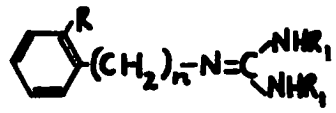
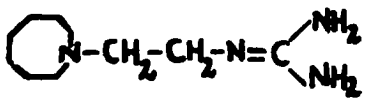
Results

Table 1 shows the effects of the guanidine derivatives on the levels of heart norepinephrine. Compounds with only a single carbon between the phenyl and the guanidine groups had only a slight effect. One of these substances, BW 392C60, actually increased the level of the catecholamine, a finding previously reported for bretylium.[8] Guanethidine and BW 247C58, compounds with two carbon atoms separating the groups, were highly active; BW 140C60, a compound with a three carbon chain, had a lower but appreciable activity.

Table 2 describes the effects of the drugs in blocking the guanethidine-induced depletion of heart norepinephrine. All of the compounds having a single carbon atom separating the phenyl group from nitrogen showed considerably more activity than bretylium. Methyl substitution in the guanidine moiety, and ortho-halide substitution in the benzene ring resulted in more active compounds;

TABLE 1

Effect of Various Guanidine Derivatives (7.5 mg/kg i.v.) on Heart Norepinephrine Levels 7 hr after Administration to Rats

				Norepinephrine levels		
				µg/g	% of normal*	Ptosis
R	n	R_1	BW number			
H	2	H	247C58	0.26; 0.26; 0.22; 0.37	26	+++
H	2	CH_3	739C60	0.59; 0.68; 0.83; 0.81; 0.81	70	0
H	3	H	140C60	0.56; 0.60; 0.51	52	+
H	1	H	200C58	0.84; 0.73; 0.89; 0.72; 0.64	70	+++
Br	1	H	59-323	0.91; 1.03; 0.99; 0.89	88	+++
H	1	CH_3	467C60	0.72; 0.71; 0.52; 0.93	70	+++
Cl	1	CH_3	392C60	1.46; 1.29; 1.39; 1.20	126	+++
 Guanethidine				0.24; 0.36; 0.14; 0.16; 0.19 0.22; 0.19; 0.18; 0.15; 0.26	18	+++

* Mean of 12 control values: 1.08 ± 0.16 S.D. µg/g norepinephrine.

BW 392C60, a compound with chlorine in the ring and methylated in both terminal nitrogens was found to be about 20 times as active as bretylium. BW 739C60, a compound with a two carbon sidechain, did not block the release of norepinephrine despite the potency of the corresponding one carbon analogue (BW 467C60).

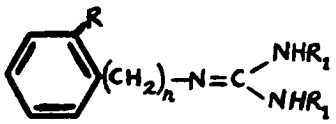
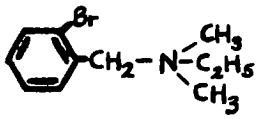
The sympatholytic action of the drugs was determined by the effects in producing ptosis (Table 1). The compounds that elicited marked ptosis were potent either in depleting norepinephrine or in blocking the depletion of norepinephrine induced by guanethidine. In contrast, only slight ptosis was produced by BW 140C60, the compound with a three carbon chain which failed to deplete norepinephrine or to block guanethidine action. Of particular interest was the time for complete ptosis to occur, 4-5 hr in the case of the norepinephrine-depleting compounds and 1-2 hr for the compounds that blocked the

depletion by guanethidine.

TABLE 2

Blocking of the Guanethidine-induced Depletion of Heart Norepinephrine by Guanidine Derivatives

Guanethidine (5 mg/kg i.v.) was given to rats 3 hr after i.p. administration of drugs. Animals were killed 5 hr later. Each value represents mean of 6 experiments.

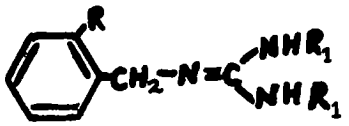
					Blockade of guanethidine-induced norepinephrine depletion*
R	n	R_1	BW number	Dose mg/kg	% of normal
Cl	1	CH_3	392C60	5	86
H	1	CH_3	467C60	5	60
H	1	CH_3	467C60	10	83
Br	1	H	59-323	5	67
Br	1	H	59-323	10	59
H	1	H	200C58	10	50
H	2	CH_3	739C60	10	0
 Bretylium				20	49

* Loss of norepinephrine 5 hr after guanethidine administration; 0.62 ± 0.06 S.D. μg/g.

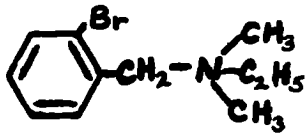
Discussion

Although the present study was carried out with a relatively small number of guanidine derivatives, the results point to certain structure-activity relationships. For example, a series of benzylguanidines exert a sympatholytic action at nerve endings similar to bretylium, a benzyl quaternary ammonium compound. In single doses, the benzylguanidines disturb transmission at peripheral adrenergic neurons by preventing the physiological release of norepinephrine. These compounds also prevent guanethidine from releasing heart nor-

epinephrine. BW 392C60, the most potent of these substances, is about 20 times more active than bretylium in preventing guanethidine-depletion, and given



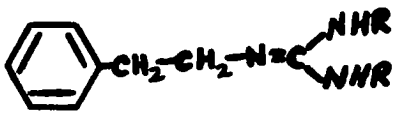
(R=H, Cl, Br; R_1=H, CH_3)
Benzylguanidines



Bretylium

alone actually causes an increase in the heart norepinephrine level. From the limited results obtained thus far, it appears that for bretylium-like activity the quaternary ammonium group is equivalent to the guanidine group and that only one carbon should separate nitrogen from the phenyl group. Activity is enhanced by halide substitution in the ortho position of the benzene ring and by methylation of both terminal nitrogens of the guanidine group.

Some phenylethylguanidines with the following general structure also exert a sympatholytic action.



(R=H)
Phenylethylguanidines

Guanethidine

These substances also affect transmission at peripheral adrenergic neurons but appear to act by depleting the amine. A previous report gives evidence supporting the view that guanethidine depletes the amine through a sustained activation of the physiological release process.[5] The most potent of these compounds, BW 247C58, is somewhat less potent than guanethidine in releasing norepinephrine from the heart. From these results, it appears that for guanethidine-like activity the benzene ring is equivalent to the 8-membered ring of guanethidine, and that two carbon atoms should separate nitrogen from the phenyl group. However, the compound with three carbon atoms also exerts some activity. In

contrast to the bretylium-like series N-methylation of the guanidine moiety results in a compound devoid of activity (BW 739C60).

In conclusion, a number of benzyl and phenylethyl analogues of guanidine and quaternary ammonium elicit similar pharmacological actions; they block transmission at peripheral adrenergic nerve terminals without antagonizing the effects of administered catecholamines. However, two different mechanisms may be involved. Compounds, such as bretylium, prevent the normal physiological release of norepinephrine as well as that induced by guanethidine; others such as guanethidine, deplete norepinephrine perhaps by persistent stimulation of the process normally activated by nerve impulses.

Compounds having a strongly basic group (guanidine or a quaternary ammonium) separated from a benzene ring by one carbon atom appear to exert a bretylium-like effect; in contrast a strongly basic nitrogen separated from a ring by two carbons appears to elicit a guanethidine-like effect.

Definitive conclusions cannot be made before these studies are extended to a larger number of compounds. However, the results indicate the importance of defining the precise nature of the biological action in relating chemical structure to activity in a meaningful way.

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