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Ketanserin Reduces a Particular Monoamine Pool in Peripheral Tissues

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SUMMARY

The effect of ketanserin and tetrabenazine treatment on monoamine and metabolite levels in central and peripheral tissues was investigated in young and senescent male Wistar and spontaneously hypertensive Okamota rats. Control animals showed significantly higher brain monoamine levels and 3 and 5.5 times higher dopamine levels in the vas deferens of the senescent and hypertensive rats, as compared with young normotensive rats. Ketanserin (20 mg/kg) produced an average of 20% reduction of brain monoamines without changing metabolite levels. In the vas deferens, dopamine was reduced by 85% and norepinephrine by 30%. In cardiovascular tissues, norepinephrine was 40% to 50% decreased and in the spleen norepinephrine was 60% and 5-hydroxytryptamine 30% reduced. Ketanserin (5 mg/kg) had only a marked effect on dopamine in the vas deferens and on norepinephrine in the portal vein. Tetrabenazine at 20 mg/kg produced complete depletion of the monoamine and 3-methoxytyramine levels in the brain with a concomitant rise in acid metabolites. In peripheral tissues, amine levels were reduced by 55% to 80%; dopamine in the vas deferens was 93% decreased. Tetrabenazine (5 mg/kg) still had marked effects in all tissues. The drug effects were the same in the three types of rats and the effects did not markedly change with chronic treatment up to 20 days. It is hypothesized that at least two different mechanisms are involved in monoamine depletion, 1) the classically proposed inhibition of uptake of monoamines in the storage vesicles, a property of tetrabenazine not shared by ketanserin *in vivo* and 2) triggering of the release of monoamines from a ketanserin-sensitive pool, which is relatively more important in peripheral tissues than in the brain. The latter process is probably mediated by previously identified ketanserin-binding release sites on nerve terminals and platelets. The ketanserin-sensitive monoamine pools in peripheral tissues may have a role in cardiovascular pathologies.

Ketanserin has become a prototype of a potent 5-HT_2 antagonist in pharmacological, radioligand binding, and clinical studies (1). Besides very potent serotonin antagonism, the drug reveals less potent α_1-adrenergic and histamine-H_1 antagonism (2). A demonstrated amplification mechanism between 5-HT_2 and adrenergic receptor blockade has been suggested to underlie its antithrombotic and antivasoconstrictive activity, leading to beneficial effects on pathologies caused by impaired blood circulation (1, 3).

[^{3}H]Ketanserin is the most widely used ligand to label 5-HT_2 receptors (4). However, in the striatum and in platelets of various mammalian species, [^{3}H]ketanserin was found to label nonserotonergic, saturable, binding sites (5, 6). Recently, these sites have been identified; they occur on catecholaminergic and serotonergic nerve terminals and on platelets and have a role in the release of monoamines and their acid metabolites from brain and peripheral tissues *in vitro* (7, 8). Ketanserin and the monoamine-depleting agent tetrabenazine (9) bind with nanomolar affinity, and reversibly, to these sites; the monoamine-depleting drug reserpine interacts with them in a nonreversible way. Drugs that interacted with these sites were found to trigger the release of catecholamines, 5-HT, and their acid metabolites from brain slices and platelets, with potencies corresponding to their binding affinities. Only a few structural congeners of ketanserin and tetrabenazine showed these properties; the interaction with the 'release sites' appeared to be unrelated to interaction with the commonly known neurotransmitter receptor sites or the transporters involved in the uptake of the monoamines in synaptosomes or platelets (7, 8).

In view of these recently discovered *in vitro* properties of ketanserin, which it has in common with tetrabenazine, we have now investigated the effect of ketanserin and tetrabenazine treatment on the levels of monoamines and metabolites in the brain and in peripheral tissues of young, senescent, and spontaneously hypertensive rats.

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; NE, norepinephrine; E, epinephrine; MOPEG, 1-(4-hydroxy-3-methoxy)phenyl-1,2-ethanediol; DA, dopamine; DOPAC, 3,4-dihydroxybenzene acetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindole acetic acid; HPLC, high-performance liquid chromatography; BID, bis in diem; IP, intraperitoneally.

Experimental Procedures

Young male Wistar rats (200 g, age 2 months), senescent male Wistar rats (age 7–11 months), and spontaneously hypertensive male Okamota rats (200 g, age 2 months) were acutely or repeatedly treated IP with ketanserin or tetrabenazine; dosages and duration of treatment are indicated in the tables and in the figure. Control rats received saline injections. The animals were killed by decapitation 2 hr after the last injection. Brain and peripheral tissues were rapidly dissected, immediately weighed, and frozen in liquid nitrogen (time between killing and freezing of the tissue was less then 5 min). The tissues were lyophilized for 24 hr and stored at −80° until analysis. The tissue content of monoamines NE, DA, and 5-HT and of monoamine metabolites DOPAC, HVA, 3-MT, and 5-HIAA was determined using HPLC. After precise weight determination, the dry tissue was extracted in 1 to 10 ml of 0.4 M perchloric acid, as previously described (10). Forty microliters of the acid extract was injected in an HPLC apparatus (Varian HPLC model 5060) equipped with a microcomputer-controlled reciprocating single piston pump system. The electrochemical detector was a BAS model 4B from Bioanalytical Systems (West Lafayette, IN) equipped with a glassy carbon electrode. Experimental conditions were as described previously (10).

Relative retention times, related to serotonin, and minimum detectable quantity were as follows: NE, 0.17, 1.8 pg; MOPEG, 0.19, 1.7 pg; E, 0.22, 2.2 pg; DOPAC, 0.27, 1.7 pg; DA, 0.38, 1.4 pg; 5-HIAA, 0.50, 1.0 pg; HVA, 0.60, 3.5 pg; 3-MT, 0.90, 18 pg; and 5-HT, 1.0, 1.6 pg. The absolute retention time of 5-HT was 25 min. Ketanserin-tartaric acid salt (R 49 945, Janssen Research Foundation, Beerse, Belgium) and tetrabenazine (Hoffmann-La Roche, Basel, Switzerland) were dissolved in saline.

Results

Control monoamine and metabolite levels in brain areas and peripheral tissues of young normotensive male Wistar rats (age 2 months), of senescent male Wistar rats (age 7–11 months), and of spontaneously hypertensive Okamota rats (age 2 months) are shown in Table 1. Each tissue was analysed for the complete spectrum of monoamines and metabolites but only relevant data, i.e., when significant amounts were detectable, are shown. The monoamine levels in the brains of the senescent and hypertensive rats (except those of 5-HT in the striatum of the latter) were significantly higher than in the young normotensive rats. The DA pool in the vas deferens of the senescent and the hypertensive rats was 3 and 5.5 times higher, respectively, and the NE pool in this tissue was significantly lower, than in the young normotensive rats.

Drug treatment in young normotensive rats. Young male Wistar rats (age 2 months) were acutely treated with 5 or 20 mg/kg ketanserin and tetrabenazine; control animals received saline. Two hours later, the frontal cortex, striatum, vas deferens, spleen, left ventricle, caudal artery, and portal vein were dissected and prepared for HPLC analysis. The data on the content of NE, DA, the DA metabolites DOPAC, HVA, and 3-MT, and 5-HT and its metabolite 5-HIAA, expressed as percentage of control levels, are presented in Table 2. With each series of experiments, control tissues were run; the measured content of the various substances was highly reproducible, as is apparent from the mean values ± standard deviations indicated in Table 1.

As shown in Table 2, 20 mg/kg ketanserin caused a significant reduction of 20% to 37% of the monoamines in the frontal cortex and of 22% of the DA in the striatum. 5-HT levels were less affected (not significantly in the striatum); NE was most reduced. The metabolites, 3-MT, DOPAC, and 5-HIAA, were not changed; HVA in the striatum was slightly but significantly increased. In the peripheral tissues, a larger reduction in monoamine levels was observed. NE was reduced by 31% in the vas deferens, by 44% in cardiovascular tissues, and by 66% in the spleen. 5-HT was 29% decreased in the spleen. A conspicuous reduction of 85% in DA in the vas deferens was noted. Tetrabenazine (20 mg/kg) caused a total depletion of NE, DA, and 3-MT in the brain tissues; 5-HT was 85% reduced and the brain content of 5-HIAA, DOPAC, and HVA was increased by about 170%, 200% to 300% and more than 400%, respectively. In contrast to ketanserin, tetrabenazine caused a less pronounced depletion of monoamines in the peripheral tissues, as compared with central tissues. NE levels were most reduced in

TABLE 1

Control monoamine and metabolite levels in brain areas and peripheral tissues of young, senescent, and hypertensive rats

Values are the average ± standard deviation of measurements in separate tissues from the number of rats shown in parentheses.

Tissue	Compound	Male Wistar, 2 months (A)	Male Wistar, 7–11 months (B)	Male Okamota, 2 months (C)
		pmol/mg of dry weight		
Frontal cortex	NE	7.7 ± 2.0 (32)	8.8 ± 2.7 (11)	7.1 ± 1.8 (15)
	DA	1.6 ± 0.5 (34)	3.1 ± 1.4 (11)[a]	2.6 ± 0.6 (15)[a]
	DOPAC	0.39 ± 0.23 (34)	0.38 ± 0.29 (11)	0.34 ± 0.27 (15)
	5-HT	11.5 ± 1.7 (34)	19.8 ± 5.9 (11)[a]	14.4 ± 1.6 (15)[a]
	5-HIAA	5.6 ± 0.9 (35)	7.4 ± 1.4 (11)[a]	5.9 ± 0.7 (15)
Striatum	DA	311 ± 46 (35)	372 ± 59 (11)[a]	350 ± 46 (16)[b]
	DOPAC	32.1 ± 5.3 (33)	30.6 ± 5.7 (11)	34.2 ± 6.8 (16)
	HVA	20.0 ± 4.8 (35)	22 ± 5 (11)	17.5 ± 2.8 (16)[c]
	3-MT	9.9 ± 2.9 (35)	10 ± 2 (11)	8.6 ± 4.0 (16)
	5-HT	8.4 ± 1.4 (36)	11.8 ± 4.0 (11)[b]	8.5 ± 1.5 (16)
	5-HIAA	10.1 ± 1.5 (35)	12.3 ± 2.9 (11)[c]	10.1 ± 1.6 (16)
Vas deferens	NE	322 ± 74 (35)	223 ± 39 (11)[a]	236 ± 48 (16)[a]
	DA	8.6 ± 3.4 (34)	28.2 ± 7.3 (11)[a]	47.8 ± 14.2 (16)[a]
Spleen	NE	17.6 ± 6.5 (17)		
	5-HT	41.5 ± 10.2 (16)		
Left ventricle	NE	16.1 ± 4.9 (24)		
Caudal artery	NE	76.7 ± 36.4 (23)		
Portal vein	NE	76.6 ± 19.0 (23)		

Statistic analysis was according to Student's *t* test (two-tailed). Significance of difference between group A and B or C, respectively: [a] $p \leq 0.001$; [b] $p \leq 0.01$; [c] $p \leq 0.05$; no indication, $p > 0.05$.

TABLE 2

Monoamine and metabolite levels in tissues of young male Wistar rats, 2 hr after IP treatment with ketanserin or tetrabenazine

Rats were 2 months old. The experiments were designed such that with each series of two to four treated animals an equal number of controls was run in parallel. Percent values were calculated versus these matched controls and then averaged. The average control values are shown in Table 1. Values are mean ± standard deviation from the number of measurements shown in parentheses.

Tissue	Compound	Ketanserin		Tetrabenazine	
		5 mg/kg	20 mg/kg	5 mg/kg	20 mg/kg
		% of matched controls			
Frontal cortex	NE	85 ± 17 (8)[c]	63 ± 11 (5)[a]	31 ± 14 (8)[a]	0 (5)[a]
	DA	113 ± 27 (7)	71 ± 21 (5)[a]	60 ± 24 (6)[b]	0 (5)[a]
	DOPAC	123 ± 31 (4)	83 ± 61 (5)	147 ± 28 (4)[c]	283 ± 55 (5)[a]
	5-HT	86 ± 8 (8)[a]	80 ± 9 (5)[a]	46 ± 18 (6)[a]	12 ± 3 (5)[a]
	5-HIAA	95 ± 12 (8)	104 ± 14 (5)	138 ± 19 (8)[a]	169 ± 24 (5)[a]
Striatum	DA	96 ± 13 (8)	78 ± 12 (6)[a]	15 ± 6 (8)[a]	4 ± 1 (6)[a]
	DOPAC	94 ± 9 (8)	103 ± 25 (6)	198 ± 21 (8)[a]	216 ± 22 (6)[a]
	HVA	78 ± 15 (8)[b]	134 ± 19 (6)[a]	265 ± 36 (8)[a]	434 ± 79 (6)[a]
	3-MT	73 ± 29 (8)[c]	95 ± 44 (6)	21 ± 15 (7)[a]	0 (6)[a]
	5-HT	94 ± 21 (8)	88 ± 17 (6)	50 ± 11 (7)[a]	17 ± 6 (6)[a]
	5-HIAA	91 ± 23 (8)	107 ± 24 (6)	140 ± 20 (8)	188 ± 49 (6)[a]
Vas deferens	NE	89 ± 10 (7)[b]	69 ± 15 (6)[a]	73 ± 7 (7)[a]	33 ± 10 (6)[a]
	DA	38 ± 12 (7)[a]	15 ± 4 (6)[a]	20 ± 5 (8)[a]	7 ± 3 (6)[a]
Spleen	NE	93 ± 26 (8)	34 ± 22 (8)[a]	43 ± 9 (7)[a]	8 ± 4 (8)[a]
	5-HT	95 ± 15 (7)	71 ± 20 (8)[a]	53 ± 14 (8)[a]	46 ± 11 (8)[a]
Left ventricle	NE	90 ± 22 (8)	57 ± 10 (8)[a]	62 ± 14 (8)[a]	38 ± 19 (8)[a]
Caudal artery	NE	124 ± 40 (8)	61 ± 6 (8)[a]	62 ± 37 (8)[b]	22 ± 5 (8)[a]
Portal vein	NE	72 ± 12 (4)[b]	56 ± 8 (8)[a]	40 ± 6 (4)[a]	11 ± 6 (8)[a]

Statistic analysis was according to the Student *t* test (two-tailed). Significance of difference from controls: [a] $p \leq 0.001$; [b] $p \leq 0.01$; [c] $p \leq 0.05$; no indication, $p > 0.05$.

the spleen (92%) and least in the left ventricle (62%). 5-HT in the spleen was only reduced by 54% but, as with ketanserin, a very marked decrease of DA in the vas deferens was observed.

At a dose of 5 mg/kg, ketanserin had only minor effects on monoamine or metabolite levels in the brain, whereas tetrabenazine caused a marked decrease of monoamine and 3-MT levels and an increase in the levels of the acid metabolites. In the peripheral tissue, 5 mg/kg ketanserin produced a significant reduction of 28% of the NE content in the portal vein and a reduction of 62% of DA in the vas deferens. Tetrabenazine (5 mg/kg) decreased the latter by 80%. At the low dose, tetrabenazine was again less active on the NE content in the peripheral tissues, as compared with the brain. However, the 5-HT content was equally reduced by 50% in the brain and the peripheral tissues. It was noted that 5 and 20 mg/kg tetrabenazine produced the same reduction of 50% of the 5-HT content in the spleen (Table 2).

Fig. 1 shows the effect of chronic treatment of young normotensive male Wistar rats with ketanserin and tetrabenazine, 10 mg/kg IP twice daily for 6, 13, and 20 days. It appeared that the drug effects on the monoamines in the brain tissues and in the vas deferens did not significantly change with time of treatment, as compared with the acute effects. The increase in acid metabolites caused by tetrabenazine was lower after 6 and 13 days of treatment and was thereafter maintained at that level. There was no gradual time-dependent attenuation of the effect.

Drug treatment in senescent rats. Male Wistar rats, 7 to 11 months old, were acutely (20 mg/kg IP) and repeatedly, for 6 days (10 mg/kg IP BID), treated with ketanserin and tetrabenazine or saline. Two hours after the last injection, the frontal cortex, striatum, and vas deferens were prepared for analysis of the monoamine and metabolite content, as described above. Data are presented in Table 3. The findings were very similar to the findings in young rats. The drug effects were practically the same, except that in these animals ketanserin produced a slight enhancement of acid metabolites and a reduction of 15% of 3-MT in the striatum. Note also that the larger DA pool in the vas deferens of these animals was reduced by more than 90%. In the rats repeatedly treated for 6 days with ketanserin, the reduction of monoamines in the frontal cortex and of DA in the vas deferens was somewhat less than in the acutely treated rats. This could be due to the fact that the bolus dose of ketanserin during the repeated treatment was only half the dose of the acute treatment. There was no difference between repeated and acute treatment with tetrabenazine.

Drug treatment in spontaneously hypertensive rats. Spontaneously hypertensive Okamota rats (age 2 months) were similarly treated and the tissues were analyzed as described above for senescent rats. Data are presented in Table 4. The effect of ketanserin on NE in the frontal cortex and on DA in the striatum was significantly less in the Okamota rats, as compared with young and senescent male Wistar rats. Also, the central effects of tetrabenazine were somewhat less marked in the Okamota rats. However, the NE pool and the larger DA pool in the vas deferens of Okamota rats were reduced by the drugs to the same extent as in young and senescent male Wistar rats. Repeated drug treatment did not have significantly different effects than did acute treatment, except for a somewhat lower effect on the DA pool in the vas deferens of the repeatedly treated animals, which may be due to the difference in bolus dose (see above).

Discussion

In this study, we showed that ketanserin, at a dose of 20 mg/kg, produced a partial reduction of monoamine levels. The effect was more pronounced in most peripheral tissues than in the brain. The DA pool in the vas deferens was almost completely depleted, the NE content in the vas deferens, cardiovascular tissues, and the spleen was reduced by 31%, 44%, and 66%, respectively, and 5-HT in the spleen was decreased 29%. Brain catecholamines were only reduced by 20% to 37%; 5-HT

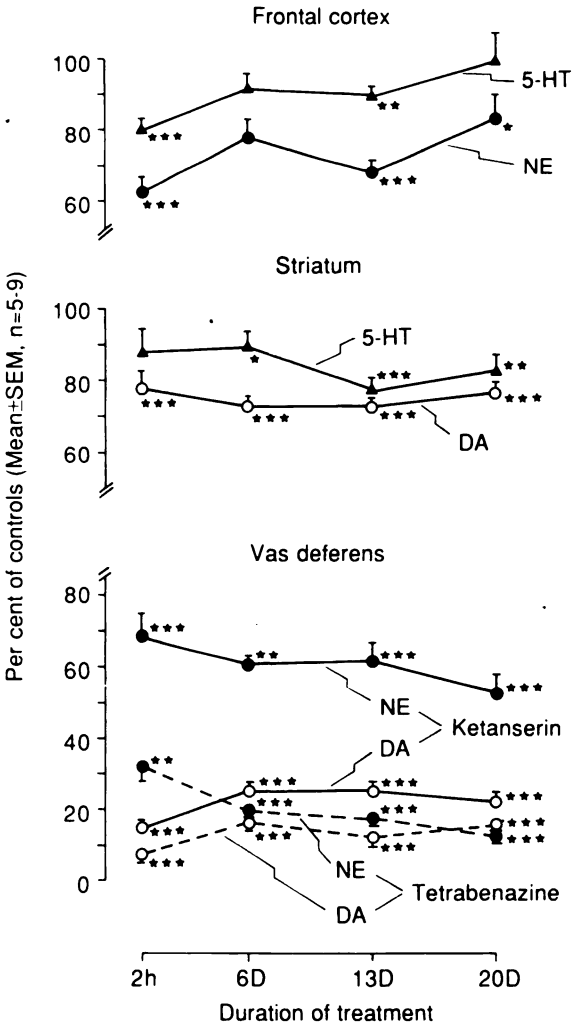

Fig. 1. Monoamine levels in rat frontal cortex, striatum, and vas deferens of young male Wistar rats treated (IP) with ketanserin or tetrabenazine. Tissues were taken 2 hr after acute (20 mg/kg) or chronic administration (10 mg/kg, BID) for 6, 13, and 20 days (D). For the frontal cortex and the striatum, findings after ketanserin treatment are shown. After tetrabenazine treatment, the monoamine levels in the brain areas were completely depleted and the levels of DOPAC, HVA, and 5-HIAA were increased (not shown on the figure). For the vas deferens, findings with both drugs are shown. Experimental design, calculations, and statistics were as described in the legend to Table 2.

was even less affected. Ketanserin at 5 mg/kg had much less effect. Tetrabenazine, which is known as a monoamine-depleting agent, showed a different profile of effects than did ketanserin. In contrast to ketanserin, tetrabenazine exerted its greatest effect on brain monoamine levels, which, with 20 mg/kg, were completely depleted, whereas the levels in most peripheral tissues were less affected. As a result, the effect of ketanserin and tetrabenazine differed less in some peripheral tissues; a similar extensive decrease of DA levels in the vas deferens was noted. The effect of ketanserin and tetrabenazine did not markedly change with time of treatment; hence, an accumulative effect of the drugs was not observed. Differences in the control levels of monoamines in young normotensive, senescent, and spontaneously hypertensive rats were noted. In particular, the 3- and 5.5-fold higher DA levels in the vas deferens of the latter rats, respectively, was conspicuous. However, the drug effects were very similar in the three kinds of rats.

The findings described here have implication i) for the interpretation of the mechanism of the monoamine-depleting action of ketanserin and tetrabenazine and ii) for the interpretation of the ketanserin effects on cardiovascular pathologies in humans.

Mechanism of the monoamine-depleting action of ketanserin and tetrabenazine. Tetrabenazine and reserpine have long been thought to cause depletion of monoamines by inhibiting the uptake of the amines in the storage vesicles (9, 11–13). The drugs presumably block the monoamine transporter, which occurs on synaptic vesicles and chromaffin granules. Scherman *et al.* (14–16) have studied the labeling of the transporter with [^{3}H]reserpine and [^{3}H]dihydrotetrabenazine. Tetrabenazine revealed high affinity, reversible binding, in contrast to reserpine, which bound quasi-irreversibly. As in the present study, it has previously been observed that tetrabenazine had a more marked monoamine-depleting action in the brain than in the periphery. This has been ascribed to the existence of a 'fast pool' in the brain and a 'slow pool' in the periphery (14–16).

The question is whether monoamine depletion in the brain and in the periphery occur via a single common mechanism, i.e., prevention of the storage of the amines in vesicles, and whether both ketanserin and tetrabenazine act via this mechanism. As described above, we have recently identified high affinity binding sites for ketanserin in brain membrane preparations and on platelets, to which tetrabenazine also binds with high affinity. The sites are associated with catecholaminergic and serotonergic nerve endings and have a role in the release of monoamines and their acid metabolites from rat brain slices and of 5-HT from human platelets. The release of unmetabolized monoamines was particularly apparent in the platelets; in the brain, it was seen in the presence of a monoamine oxidase inhibitor (7, 8). The time course of the effect in the *in vitro* studies suggested that the release-evoking effects of ketanserin- and tetrabenazine-like drugs, and the associated ketanserin binding sites, occurred at the level of the plasma membrane. After our findings had been disclosed, before publication, to a group of French investigators, they studied the mutual interaction of ketanserin derivatives with [^{3}H]dihydrotetrabenazine binding sites on a chromaffin granule membrane preparation. The drugs were found to bind to the same site that was hypothesized to be the monoamine transporter (17). This conclusion was largely based on previous studies on the identification of the [^{3}H]dihydrotetrabenazine binding sites (14, 16). Unfortunately, the identity of the sites could never be verified by others because of the lack of availability of the labeled dihydrotetrabenazine.

Based on the findings in this study, we hypothesize that at least two mechanisms are involved in the depletion of the monoamines *in vivo*. Tetrabenazine appears to act via both mechanisms, whereas ketanserin seems only to be active on one system *in vivo*. The first mechanism, insensitive to ketanserin *in vivo*, probably is the classically proposed inhibition of the uptake of the monoamines in the storage vesicles. This mechanism apparently is predominant in the brain and responsible for the extensive depletion of central monoamines by tetrabenazine, an effect that is not obtained with ketanserin. The second mechanism is release of the monoamines from a ketanserin-sensitive pool. This pool appears to be relatively more important in peripheral tissues. A distinction in sensitiv-

TABLE 3

Monoamine and metabolite levels in brain areas and the vas deferens of senescent male Wistar rats, 2 hr after acute or 6-day IP treatment with ketanserin or tetrabenazine

Rats were 7 to 11 months old. Experimental design, calculations, and statistics were as described in the legend to Table 2.

Tissue	Compound	20 mg/kg acutely		10 mg/kg BID for 6 days	
		Ketanserin	Tetrabenazine	Ketanserin	Tetrabenazine
		% of matched controls			
Frontal cortex	NE	65 ± 15 (8)[a]	1 ± 2 (6)[a]	78 ± 12 (8)[a]	0 ± 1 (4)[a]
	DA	72 ± 10 (8)[a]	16 ± 9 (6)[a]	94 ± 42 (8)	15 ± 19 (4)[a]
	DOPAC	107 ± 50 (8)	248 ± 109 (6)[b]	89 ± 61 (8)	341 ± 161 (4)[b]
	5-HT	76 ± 11 (8)[a]	10 ± 3 (6)[a]	85 ± 14 (8)[c]	9 ± 3 (4)[a]
	5-HIAA	115 ± 25 (8)	203 ± 18 (6)[a]	112 ± 19 (8)	175 ± 18 (4)[a]
Striatum	DA	87 ± 14 (8)[c]	5 ± 1 (6)[a]	80 ± 12 (8)[a]	4 ± 2 (4)[a]
	DOPAC	122 ± 35 (8)[c]	248 ± 43 (6)[a]	145 ± 38 (8)[b]	166 ± 28 (4)[a]
	HVA	143 ± 53 (7)[c]	383 ± 49 (6)[a]	219 ± 94 (8)[b]	264 ± 54 (4)[a]
	3-MT	84 ± 13 (8)[c]	9 ± 5 (6)[a]	84 ± 50 (8)	5 ± 8 (4)[a]
	5-HT	84 ± 14 (8)[c]	15 ± 4 (6)[a]	80 ± 17 (8)[b]	7 ± 2 (4)[a]
	5-HIAA	111 ± 23 (8)	168 ± 21 (6)[a]	119 ± 24 (8)[c]	170 ± 20 (4)[a]
Vas deferens	NE	74 ± 8 (8)[a]	42 ± 4 (6)[a]	58 ± 17 (7)[a]	20 ± 3 (4)[a]
	DA	9 ± 2 (8)[a]	3 ± 1 (6)[a]	25 ± 4 (8)[a]	12 ± 5 (4)[a]

Significance of difference from controls: [a] $p \leq 0.001$; [b] $p \leq 0.01$; [c] $p \leq 0.05$; no indication, $p > 0.05$.

TABLE 4

Monoamine and metabolite levels in brain areas and the vas deferens of spontaneously hypertensive male Okamota rats, 2 hr after acute or 6-day IP treatment with ketanserin and tetrabenazine

Experimental design, calculations, and statistics were as described in the legend to Table 2.

Tissue	Compound	20 mg/kg acutely		10 mg/kg BID for 6 days	
		Ketanserin	Tetrabenazine	Ketanserin	Tetrabenazine
		% of matched controls			
Frontal cortex	NE	86 ± 10 (8)[b]	3 ± 4 (8)[a]	75 ± 16 (8)[a]	1 ± 2 (8)[a]
	DA	88 ± 22 (8)	21 ± 6 (8)[a]	89 ± 26 (8)	22 ± 6 (8)[a]
	DOPAC	97 ± 31 (8)	233 ± 77 (8)[a]	55 ± 75 (8)	169 ± 26 (8)[a]
	5-HT	91 ± 9 (8)[c]	25 ± 6 (8)[a]	93 ± 15 (8)	25 ± 8 (8)[a]
	5-HIAA	106 ± 16 (8)	213 ± 27 (8)[a]	110 ± 12 (8)[c]	188 ± 25 (8)[a]
Striatum	DA	92 ± 7 (8)	7 ± 3 (8)[a]	85 ± 10 (8)[a]	7 ± 2 (8)[a]
	DOPAC	94 ± 17 (8)	223 ± 44 (8)[a]	85 ± 10 (8)[b]	162 ± 30 (8)[a]
	HVA	132 ± 23 (8)[a]	446 ± 76 (8)[a]	108 ± 19 (8)	266 ± 50 (8)[a]
	3-MT	92 ± 41 (8)	12 ± 17 (8)[a]	86 ± 33 (8)	12 ± 9 (8)[a]
	5-HT	93 ± 9 (8)	25 ± 8 (8)[a]	87 ± 15 (8)[c]	26 ± 7 (8)[a]
	5-HIAA	101 ± 10 (8)	168 ± 15 (8)[a]	101 ± 17 (8)	164 ± 20 (8)[a]
Vas deferens	NE	68 ± 12 (8)[a]	38 ± 11 (8)[a]	70 ± 5 (8)[a]	15 ± 3 (8)[a]
	DA	15 ± 4 (8)[a]	4 ± 1 (8)[a]	30 ± 9 (8)[a]	12 ± 3 (8)[a]

Significance of difference from controls: [a] $p \leq 0.001$; [b] $p \leq 0.01$; [c] $p \leq 0.05$; no indication, $p > 0.05$.

ity of the various amines is noted (DA > NE > 5-HT) and for a given amine the relative size of the pool varies with tissues. The ketanserin-sensitive pool represents 40% to 65% of the NE content in cardiovascular tissues and spleen. DA in the vas deferens seems to be primarily contained in this pool, but the NE in the vas deferens partly occurs in the ketanserin-sensitive pool (30%) and partly in the ketanserin-insensitive pool. The differential effect of ketanserin on the DA and NE levels in the vas deferens is highly suggestive of the existence of two distinct pools. Furthermore, our findings have shown that the DA-containing, ketanserin-sensitive, pool in the vas deferens becomes larger in senescent and spontaneously hypertensive rats. Based on our studies *in vitro* (see above) it can be suggested that the release of the content of the ketanserin-sensitive pool is triggered by interaction of the drugs with the release-evoking ketanserin binding sites, presumably localized on the plasma membranes. The *in vitro* studies suggest that the amines in this pool are readily metabolized by monoamine oxidase in the brain, but less so in peripheral tissues. It is not yet clear whether a similar type of binding site would be associated with the monoamine transporter on the storage vesicles, as suggested by Darchen *et al.* (17). The possibility remains that the latter intracellular sites are not sufficiently reached by ketanserin *in vivo*.

Ketanserin in humans. Ketanserin is known to have potent antithrombotic and antivasoconstrictive activities, due to concomitant blockade of 5-HT_2 and α-adrenergic receptors on platelets and vessel walls (see above). These properties are thought to underlie the beneficial effects of ketanserin in cardiovascular pathologies, but they could not explain the entire profile of activities of the drug. It has been proposed that a central sympathoinhibitory action, probably mediated by α_1-receptor blockade, could be involved (3, 18). The sympathoinhibitory action of ketanserin has recently been corroborated, and evidence was provided that it probably played a role in the ketanserin-induced bradycardia and hypotension (19). The presently described partial monoamine-depleting effects may provide an additional explanation for certain actions of ketanserin. The partial monoamine-depleting activity of ketanserin has been confirmed in studies in humans, in which a reduction by about 20% of platelet-bound 5-HT has been observed after

ketanserin treatment at therapeutic dosages.[1] Our observation, in laboratory animals, that the ketanserin-sensitive monoamine pool becomes more important in hypertensive and in old animals could be related to the clinical observation that ketanserin is particularly effective in elderly patients with essential hypertension (20–23). The demonstrated preferential activity in peripheral tissues on a particular ketanserin-sensitive monoamine pool distinguishes the action of ketanserin from that of tetrabenazine- and reserpine-like drugs and make noxious side effects, known for the latter, unlikely. This newly discovered activity of ketanserin may provide a new lead for the exploration of the pathogenesis of various cardiovascular diseases.

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