Evidence for Rapid Turnover of Norepinephrine in Rat Heart and Brain. (27710)

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Decreased levels of norepinephrine in brain and heart have been observed in rats and other species following administration of several different compounds. These include rauwolfia alkaloids(1), benzoquinolizines(2,3), and certain aromatic α-methyl-amino acids and amines(4,5). Whenever depletion of norepinephrine is marked (>75%), the reduced concentrations have been noted to persist for longer than 24 hours. This has been interpreted as indicating either slow turnover of the amine or a prolonged interference with storage mechanisms.

During investigations on catecholamine-indole relationships it was found in this laboratory that 1-(5,6 dimethoxy-2-methyl-3 indole)-ethyl-4-phenylpiperazine (Win 18501-2, oxypertine) decreases norepinephrine in heart and brain without affecting brain serotonin. The reduction of tissue levels of the catecholamine and its return to control values occurred within a period of 8 hours. Win 18501-2 is from a series of 1-(indolyl-alkyl)-4-arylpiperazines described by Wylie and Archer(6), which cause central nervous system depression. Significance of these findings in terms of turnover rate of norepinephrine and mechanism of the drug's depressant action is presented.

Materials and methods. Male rats of Sprague-Dawley strain, 200-250 g body weight, were used in these studies. Single intraperitoneal injections of Win 18501-2, 60 mg/kg were given. The compound was dissolved in propylene glycol. Control animals received the same volume of the solvent. At various times after drug administration the animals were sacrificed, tissues rinsed with water, blotted on filter paper and analyzed for serotonin and norepinephrine by methods previously described(7,8).

Some animals were also given intraperitoneal injections of the monoamine oxidase inhibitor N-methyl-N-benzyl-propargylamine (MO-911) or bretylium tosylate dissolved in isotonic NaCl in order to study the mechanism by which Win 18501-2 produced norepinephrine depletion.

Results. The changes in heart and brain norepinephrine content in animals receiving Win 18501-2 are summarized in Fig. 1. The norepinephrine content of the heart was lowered from an average level of 0.9 to 0.14 μg/g within 3 hours. This decrease is comparable to that produced by 1 mg/kg of reserpine. The levels remained low for another hour, returned to 50% of normal at 6 hours, and attained values which were 80-90% of control levels after 8 hours. As shown, the norepinephrine content of the brain also declined, but by only 60% in 3 hours. In contrast, the serotonin levels in the brain were found to be unaltered from control values (0.65 ± 0.08 μg/g) during the entire time course. As reported previously(6) administration of Win 18501-2 caused obvious CNS depression within 5 min which persisted for about 12 hours.

Pretreatment of the rats with the monoamine oxidase inhibitor MO-911 (75 mg/kg) prevented the decline in norepinephrine concentrations in heart and brain (Table I). The CNS depression produced by Win 18501-2 was not reversed by pretreatment with the monoamine oxidase inhibitor. When Win 18501-2 (60 mg/kg) was given 1 hour before MO-911 (75 mg/kg) and the animals were killed 3 hours after MO-911, the level of norepinephrine in brain was found to be normal and the norepinephrine concentration in the heart was slightly below normal (Table I). Kuntzman *et al.*(9) reported that bretylium (20 mg/kg) twice daily for 7 days blocked guanethidine-induced release of norepinephrine in the heart. In similar studies we found that treatment with bretylium failed to prevent the release of heart norpinephrine induced by 60 mg/kg Win 18501-2 (Table I).

Discussion. The return of heart norepinephrine to control values following Win 18501-

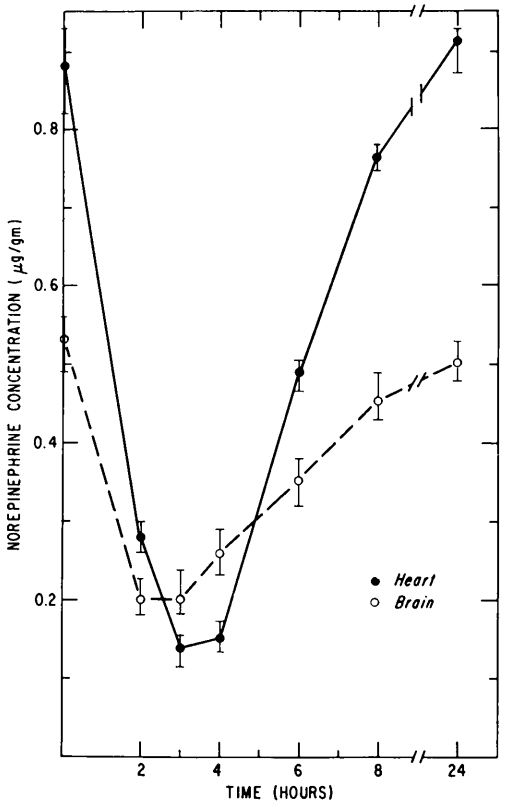

FIG. 1. Changes in norepinephrine levels of rat heart and brain after administration of single intraperitoneal dose of Win 18501-2, 60 mg/kg. Each value shown represents the avg and range of 5 experiments. In each experiment tissues from 3 animals were pooled.

2 is much faster than that with other compounds which lower the amine content of this organ to the same extent. These results suggest that the turnover of norepinephrine in the heart is rapid. Consistent with this is the report by Hess *et al.*(10) of rapid synthesis of norepinephrine in heart of reserpinized animals when the precursor substance, 3,4-dihydroxy-phenylalanine (DOPA) is administered. The data obtained with the monoamine oxidase inhibitor suggest that Win 18501-2 does not impair any of the enzymes involved in synthesis of norepinephrine. If either aromatic-L-amino acid decarboxylase or dopamine-β-oxidase were inhibited by Win 18501-2, the monoamine oxidase inhibitor should have had no effect in either maintaining or restoring the norepinephrine levels. When a dopamine-β-oxidase inhibitor is administered at a time when Win 18501-2 has caused maximum depletion of heart norepinephrine, the expected return to normal of norepinephrine levels is prevented(11).

Win 18501-2 appears to cause a release similar to that of reserpine but probably not like guanethidine, as bretylium failed to prevent the decline of heart norepinephrine. However, unlike reserpine, Win 18501-2 has a short duration of action on storage mechanisms and although it also has an indolic moiety, the compound does not alter the levels of brain serotonin. Brain norepinephrine levels are reduced and also show a rapid turnover. These findings support the concept that the storage mechanisms for the biogenic amines, serotonin, and norepinephrine, are different.

In addition to a releasing effect on tissue norepinephrine which is probably due to the indolic portion of the molecule, this com-

TABLE I. Effects of MO-911 and Bretylium on Norepinephrine Depletion Produced by Win 18501-2.

Treatment	No. Exp	Time (hr)	Norepinephrine conc'n (μg/g)§ Brain	Heart
Control	10	0	.5 (.47-.62)	.87 (.82-1.05)
Win 18501-2	6	3	.20 (.18-.24)	.15 (.11- .19)
MO-911	3	4	.86 (.76-.89)	1.0 (.98-1.17)
MO-911 + Win 18501-2	3	*	.84 (.78-.86)	1.0 (.96-1.17)
Win 18501-2 + MO-911	3	†	.80 (.72-.84)	.7 (.62- .84)
Bretylium + Win 18501-2	3	‡	.22 (.18-.25)	.19 (.15- .21)

* MO-911 admin. 1 hr prior to Win 18501-2 and animals killed 3 hr after Win 18501-2.
† Win 18501-2 admin. 1 hr before MO-911, and animals killed 3 hr after MO-911.
‡ Bretylium tosylate admin. 2 times per day for 7 days prior to Win 18501-2, animals killed 3 hr after Win 18501-2.
§ Tissues from 3 rats were pooled for each experiment.
Figures represent mean values with ranges given in parentheses.

pound also has an adrenolytic action like chloropromazine because of its phenylpiperazine moiety. Bovet *et al.*(12) have shown that 1-phenylpiperazine is weakly adrenolytic, and Archer *et al.*(13) have reported that the indole-arylpiperazines have adrenolytic properties. Thus, the CNS depression may be analogous to that ascribed to chloropromazine and unrelated to depletion of norepinephrine. The excitation exhibited by monoamine oxidase inhibited animals given either reserpine or α-methyl-meta-tyrosine has been explained by the release of norepinephrine(14). The CNS depression produced with Win 18501-2 was not reversed by pretreatment with a monoamine oxidase inhibitor. The adrenolytic properties of the compound may also account for this finding.

Summary. Administration of an indolylalkyl-arylpiperazine (Win 18501-2) to rats depletes the norepinephrine content of heart and brain without affecting brain serotonin. Maximum depletion was observed within 3 hours with return to normal values within 8 hours. These findings suggest that the turnover of heart and brain norepinephrine in the rat is very rapid.

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Tetrazolium Salts and Identification of *Corynebacterium diphtheriae.**† (27711)

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Media containing tellurite have been used extensively for detection and isolation of *Corynebacterium diphtheriae*(1,2,3) because this species reduces potassium tellurite(1,2). This is generally considered a valuable tool, even though certain other bacteria react somewhat similarly(4,5,6).

Tetrazolium salts are valuable reagents in the study of dehydrogenase systems and are superior to tellurium salts for this purpose because of their higher redox potentials(7). One of them, nitro blue tetrazolium (NO_2BT) [(-2, 2'-di-p-nitrophenyl-5, 5'-diphenyl-3, 3'-3, 3'-dimethoxy-4, 4', biphenylene) ditetrazolium chloride](8), possesses several remark-

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† The various tetrazolium salts used, with the exception of TNBT, may be purchased from Sigma Chemical Co., St. Louis, Mo. Potassium tellurite, 1% solution, was obtained from Difco Lab, Detroit, Mich.