Increased concentrations of 3-methoxy-4-hydroxyphenylethyleneglycol and homovanillic acid in rat brain after treatment with BE-2254 ("HEAT")

An accelerated turnover of catecholamines in the brain produced by neuroleptics and certain other drugs has been attributed to a compensatory increase in activity of catecholaminergic neurons in response to receptor blockade (Carlsson & Lindqvist, 1963; Laverty & Sharman, 1965; Da Prada & Pletscher, 1966; Andén, Corrodi & others, 1967; Andén, Butcher & others, 1970). The increased release and catabolism of the catecholamines leads in turn to higher brain concentrations of 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MOPEG-SO_4) or homovanillic acid (HVA), depending upon whether the receptors blocked are sensitive, respectively, to noradrenaline or to dopamine (Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965; Bartholini, Haefely & others, 1972; Meek & Neff, 1973; Keller, Bartholini & Pletscher, 1973; Braestrup, 1974). We recently suggested that BE-2254, 2-[β-(4-hydroxyphenyl)-ethylaminomethyl]-tetralone ("HEAT"), blocks receptors in the brain for both noradrenaline and dopamine, the former at somewhat lower doses than the latter (Clineschmidt, Pflueger & others, 1975). The peripheral α-adrenoceptor blocking activity of BE-2254 had previously been shown by Benthe, Gothert & Tuchinda (1972). To gain further information about the central actions of BE-2254, we have examined its effect on MOPEG-SO_4 in hypothalamic tissue and HVA in striatal tissue, as well as on the whole brain concentrations of noradrenaline and dopamine.

Female Charles River (CRCD COBs) rats, 160–200 g, were decapitated 2·5 h after treatment (i.p.) with BE-2254 or vehicle (1% methylcellulose). The animals to be used for subsequent determination of MOPEG-SO_4 or HVA were additionally treated 30 min later with probenecid (200 mg kg^{-1}, i.p.), to block efflux of the metabolites from the brain. MOPEG-SO_4 in the hypothalamus was determined according to Meek & Neff (1972), and striatal HVA according to Juorio, Sharman & Trajkov (1966). Dissection of the brain was as described by Glowinski & Iversen (1966). Catecholamines were adsorbed from neutralized perchloric acid tissue extracts on alumina and eluted with 0·1 N HCl (Anton & Sayre, 1962), then assayed fluorometrically (Porter, Totaro & Burcin, 1965).

MOPEG-SO_4 in hypothalamic tissue was elevated as a consequence of treatment with 1·25, 5 or 20 mg kg^{-1} of BE-2254, whereas HVA in striatal tissue was signifi-

Table 1. *Increase in MOPEG (hypothalamus) and HVA (striatum) following BE-2254.*

Treatment[a] (mg kg^{-1}, i.p.)	MOPEG		HVA	
	$\mu g\ g^{-1}$[b,c]	Increase %	$\mu g\ g^{-1}$ [b]	Increase %
Vehicle	0·98 s.d. 0·10		1·97 s.d. 0·10	
BE-2254 (1·25)	*1·21 s.d. 0·11	23·5	2·06 s.d. 0·24	4·6
(5)	*1·33 s.d. 0·18	35·7	*2·69 s.d. 0·32	36·5
(20)	*1·33 s.d. 0·14	35·7	*2·69 s.d. 0·26	36·5

[a] All animals received probenecid (200 mg kg^{-1}, i.p.) 30 min after treatment. Tissues were removed 2·5 h after treatment.

[b] Tissues from 5 rats were pooled for each determination; $\bar{X}$ with s.d. of 6 determinations/treatment.

[c] Expressed as the hydrogen sulphate salt.

* $P < 0{\cdot}05$ (2-tailed *t*-test).

Table 2. *Effect of BE-2254 on whole brain concentrations of catecholamines.*

Treatment[a] (mg kg^{-1} i.p.)	Noradrenaline		Dopamine	
	μg g^{-1}[b]	Decrease %	μg g^{-1}[b]	Decrease %
Vehicle	0·555 s.d. 0·033		0·768 s.d. 0·020	
BE-2254 (1·25)	0·511 s.d. 0·057	7·9	0·717 s.d. 0·063	6·6
(5)	*0·484 s.d. 0·049	12·8	*0·720 s.d. 0·049	6·3
(20)	*0·387 s.d. 0·046	30·3	*0·698 s.d. 0·037	9·1

[a] 2·5 h before removal of brain.
[b] $\bar{X}$ with s.d. of 6 rats/treatment group.
* $P < 0.05$ (2-tailed t-test).

cantly increased only by the two higher doses (Table 1). The reason why 20 mg kg^{-1} of BE-2254 was no more effective than 5 mg kg^{-1} is not apparent. At least with striatal HVA, the results cannot be attributed to a "ceiling effect", inasmuch as haloperidol (2 mg kg^{-1}) tested under similar conditions evokes about a 3-fold increase in striatal HVA (unpublished observation). Noradrenaline in the whole brain was reduced by BE-2254 in a dose-related fashion (Table 2). Smaller, but significant, reductions in dopamine were also found (Table 2).

The increase in hypothalamic MOPEG-SO_4 and striatal HVA caused by BE-2254 is consistent with the prior pharmacological studies indicating that BE-2254 blocks central receptors for both noradrenaline and dopamine (Clineschmidt & others, 1975). Also in agreement with the previous report, BE-2254 apparently affects the function of noradrenergic neurons at slightly lower doses (Table 1) and possibly to a greater extent (Table 2) than dopaminergic ones.

Although we favour interpreting the results as being indicative of receptor blockade, BE-2254 did cause a diminution of the endogenous cerebral content of catecholamines (Table 2), and, thus, a direct amine-releasing action cannot be completely dismissed. Others (Bartholini & others, 1972; Keller & others, 1973; Garattini, Bareggi & others, 1974) have suggested that similar results obtained with drugs such as clozapine, thioridazine and haloperidol might be indicative of synthesis failing to keep pace with an accelerated turnover of catecholamines.

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Influence of salt and volume on changes in rat brain angiotensin

Angiotensin II has long been known to influence central nervous system function when administered exogenously. The drug can raise systemic blood pressure in dogs, alter drinking behaviour in rats, and release antidiuretic hormone (Severs & Daniels-Severs, 1973). These are pharmacological actions, the effects of large doses of angiotensin administered to the brain via an exogenous route. Of potential physiological importance is the discovery of a complete renin-angiotensin system within the central nervous system, independent of that in the periphery (Fischer-Ferraro, Nahmod & others, 1971; Ganten, Marquez-Julio & others, 1971). These workers have found brain renin to be an isoenzyme of peripheral renin, much of the angiotensin present to be in the decapeptide form (Angiotensin I), and the highest concentrations of hormone present in the hypothalamus and brainstem, the centres of cardiovascular control and salt and water regulation.

Although much anatomic work has been done, little is known about the physiology of this hormone system in the brain. One previous report has given qualitative information on possible physiological regulation (Ganten, Granger & others, 1972). This study presents quantitative data illustrating the effects of changes in sodium balance on the concentration of brain Angiotensin I.

Male Sprague-Dawley rats (250–400 g) were housed in pairs. All animals were fed standard rat pellets and had free access to tap water except where noted. Three groups were used. Control animals were untreated; one group was given meralluride (Mercuhydrin, Lakeside) (20 mg kg^{-1}, i.m.) daily and ammonium chloride (65 mg kg^{-1}, i.p.) every other day for a total of eight days. The third group was given daily injections of desoxycorticosterone acetate in oil (Dycort, Harvey) (5 mg, i.m.) on the first day and 1 mg day^{-1} thereafter for a total of eight days. These animals had free access to 0·9% NaCl.

On the day of study, animals were anaesthetized with sodium pentobarbitone (32 mg kg^{-1}, i.p.) and bilaterally nephrectomized. After a 60 min wait (sufficient to allow for the disappearance of virtually all circulating renin (Schneider, Rostorfer & Nash, 1968), decapitation and craniotomy were performed and the brainstem between the inferior colliculi and the spinal cord was removed. This tissue was quickly