Thiol inhibition of the effects of bradykinin upon vasopressin in the toad bladder

SIR,—It seems that the antagonism of vasopressin and oxytocin by thiols is specific and that at least in part the thiol acts by directly affecting intracellular processes rather than solely by chemical reduction of the hormones (Handler & Orloff, 1964; Martin & Schild, 1965). Martin & Schild (1965) also presented evidence that the antagonism between thiols and these disulphide polypeptides is not typically competitive and may be caused by a reversible inactivation of essential disulphide groups in the receptor. Handler & Orloff (1964) have shown in addition that in the toad bladder preparation cysteine inhibits the response to theophylline (which does not contain a disulphide bond).

In view of our recent finding (Furtado, 1966; Furtado & Machado, 1966) that bradykinin inhibits the effects of vasopressin and oxytocin in the toad bladder, apparently acting as a specific and competitive antagonist, and considering the observation of Cirstea (1965) that at least some effects of bradykinin are potentiated by thiol compounds, we decided to further investigate the issue to see whether there was an interaction between bradykinin and thiols in the toad bladder.

Both bradykinin (Furtado & Machado, 1966) and the thiol compounds (Bentley, 1964; Handler & Orloff, 1964) are known to inhibit the increases due to vasopressin in the movement of water as well as the net flux of sodium across the wall of the toad bladder. The interaction of these drugs was thus examined in the isolated urinary bladder of the toad, *Bufo marinus*, using the preparation of Bentley (1958). Each of the sacs of the bilobed bladder was mounted in a chamber with Bentley-Ringer solution bathing the serosal surface, and ½-dilute Ringer within the bladder. The preparation was kept at 25 ± 1°, and at pH 7·4–7·6. The osmotic flow of water was estimated gravimetrically at 20 min intervals. The test substances were added to the serosal Ringer bath. Various doses of cysteine (cysteine hydrochloride, Eastman Kodak) (Cys) were combined with 5·0 mU/ml of vasopressin (Pitressin, Parke, Davis) (V); a single dose (1·0 μg/ml) of bradykinin (synthetic bradykinin, Sandoz) (BK) was also used. The dosage scheme can be diagrammatically represented as follows:

	Group I		Group II	
	Side A	Side B	Side A	Side B
Period I	V	V	V	V
Period II	V	V + BK (or Cys)	V + Cys	V + BK
Period III	V	V + BK + Cys	V + Cys + BK	V + BK + Cys

The control periods (side A in the group I) were used to correct for the percentage calculations because of the customary increase in response observed in applying a neurohypophysial hormone repeatedly to the bladder.

As previously observed (Furtado, 1966; Furtado & Machado, 1966) bradykinin at the dose of 1 μg/ml (1·0 mM) caused about a 50% inhibition of vasopressin (5·0 mU/ml = approximately 0·01 μM). Cysteine alone against vasopressin (0·01 μM) was ineffective at 0·5 mM; it was not until the concentration was increased to 8·0 mM that the effect of vasopressin was statistically significantly inhibited (40% inhibition). Finally, a dose of 12·0 mM cysteine promoted a 50% inhibition of the effects of vasopressin, equalling the degree of antagonism of 1 μg/ml of bradykinin (Table 1).

Fig. 1 shows that in the dose range of cysteine used there was neither summation nor potentiation of the inhibiting effects of bradykinin and cysteine upon vasopressin as far as its action on the permeability to water of the toad bladder is

TABLE 1. EFFECTS OF BRADYKININ, CYSTEINE, AND BRADYKININ + CYSTEINE ON WATER TRANSFER EVOKED BY VASOPRESSIN ACROSS THE TOAD BLADDER

V‡ + Cys (0·5)†	V + BK	V + BK + Cys (0·5)	V + Cys (1)	V + BK	V + BK + Cys (1)	V + Cys (4)	V + BK	V + BK + Cys (4)	V + Cys (8)	V + BK	V + BK + Cys (8)	V + Cys (12)	V + BK	V + BK + Cys (12)
100* (3)	55 (2)	25 (5)	95 (3)	50 (3)	85 (6)	89 (3)	60 (2)	90 (5)	61 (3)	55 (3)	60 (5)	45 (3)	40 (2)	45 (5)

‡ V = vasopressin 5·0 mU/ml;
† Cys = cysteine, dose in mM in parentheses;
BK = bradykinin 1·0 mM;
* The results presented in this horizontal line express the remaining activity of vasopressin in the presence of the drugs (Cys and/or BK) as the percentage of the response obtained with vasopressin alone in the corresponding period.
Number of experiments in parentheses.

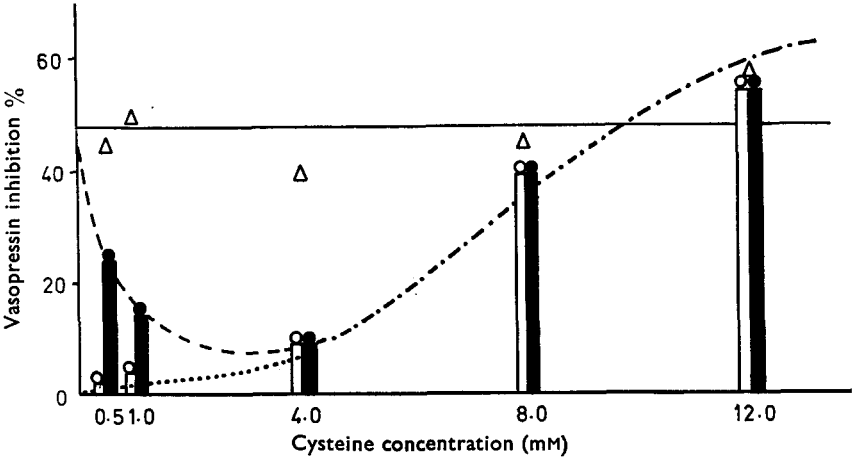

FIG. 1. Inhibition of the effects of vasopressin (5·0 mU/ml) on the permeability to water of the toad bladder by bradykinin, 1·0 mM. (△—△), cysteine in various doses (○.....○, white columns), and bradykinin + cysteine (●---●, black columns). Each point represents the mean of the experimental observations.

concerned. On the contrary, it shows that there was an inhibition of the effects of bradykinin upon vasopressin in the presence of cysteine, and this inhibition was increased by augmenting the concentration of cysteine up to 4·0 mM. By increasing the dose of thiol to 8·0 and 12·0 mM it was possible to exceed even the 50% degree of inhibition that was achieved when bradykinin was used alone against vasopressin.

In view of the present observation of inhibition of the effects of bradykinin by cysteine, both drugs being "agonists" in their having an antagonistic action upon vasopressin in the toad bladder, one may interpret the whole picture as a "competition" for a common site in the receptor. Fig. 1 shows that at low concentrations (0·5 and 1·0 mM) cysteine alone was practically ineffective but was sufficient, in a comparable dosage, to block part of the response to bradykinin, possibly by competing for a common binding site. In the presence of higher doses (4·0–12·0 mM) of cysteine the access of bradykinin to receptor sites was probably completely blocked by the thiol, and the degree of inhibition thus obtained conceivably reflected only the response due to cysteine alone (open columns with the same height as solid columns in Fig. 1).

As previously suggested (Furtado, 1966), bradykinin seems to compete with neurohypophysial hormones for the receptor site through ionic, hydrogen, and hydrophobic bonds rather than by breaking the S—S bridge of the hormones. If bradykinin and cysteine are actually competing for a common site in the receptor this fact makes it probable that thiols might also directly act at a receptor level rather than by solely reducing the hormones.

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Decrease of homovanillic and 5-hydroxyindoleacetic acids in the brain after hypothalamic lesions

SIR,—Destruction of the medial forebrain bundle (MFB) in the lateral hypothalamus decreases the 5-hydroxytryptamine (5-HT), noradrenaline and dopamine content in various parts of the rat brain (Heller & Moore, 1965; Andén, Dahlström, Fuxe & others, 1966). These changes occur not only in areas directly innervated by the axons of the MFB, e.g. the hypothalamus and limbic forebrain, but also in regions not directly connected with the MFB, possibly as a consequence of lesions of other fibres, e.g. nigro-striatal (Andén & others, 1966). The decrease of the cerebral monoamines might be due to different mechanisms, for example diminution of the storage or inhibition of the synthesis of the amines. The finding of a diminished activity of decarboxylase of aromatic amino-acids after lesion of the MFB reported by Heller, Seiden, Porcher & Moore (1965) does not necessarily indicate an impaired synthesis of catecholamines and 5-HT, since decarboxylation of 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan do not seem to be a limiting step in the biosynthesis of these amines (Hess, Connamacher, Ozaki & Udenfriend, 1961). By influencing the cerebral content of homovanillic and 5-hydroxyindoleacetic acids, the major metabolites of dopamine and 5-HT respectively, further information might be gained on the mechanism which leads to a decrease of the aromatic monoamines. Therefore these acids, as well as the chlorpromazine-induced increase of homovanillic acid, were measured in the basal parts of each brain side after unilateral lesions of the MFB. Chlorpromazine was chosen since the drug enhances the hydroxylation of tyrosine *in vivo* and increases the formation of homovanillic acid in the brain possibly as a consequence of a primary blockade of dopaminergic receptors (Andén, Roos & Werdinius, 1964; Burkard, Gey & Pletscher, 1967).