employed in the present study have been previously characterized according to growth pattern and histology by GARDNER[9]. The granulosa (Types I and III) and theca tumors (Type II) described by GARDNER were usually composed of at least 70–80% of the particular cell type designated. In addition, the tumors were functional having been shown to produce estrogen, and to a lesser extent, androgen. It was noted by LI and GARDNER[10,11] that the ovaries of tumor bearing mice treated with gonadotrophin (pregnant mare serum) showed androgenic effects. The ovarian suppression by the theca cell tumors noted in the present study further attest to the androgenic effects produced by this tumor type. Thus, the histological and endocrine aspects of these tumors are well established.

Previous investigations have implicated the theca cells and their derivatives, the interstitial cells, as an index of HCG sensitivity[12]. In the hypophysectomized rat, HCG has been reported to exert a powerful stimulating effect on the ovarian interstitial cells[2]. In the intact animal, HCG is known to promote follicular growth and luteinization due to its synergistic activity with the pituitary gonadotrophins[13]. The receptor cell for HCG in the pseudopregnant rat ovary is reportedly the lutein cells of the corpus lutea[14]. Thus, it appears that most of the ovarian cellular constituents are sensitive to HCG stimulation depending on the physiological state of the animal ovary.

The two cell theory of hormone production in the ovary states that the granulosa cells produce one type of steroid, the theca another, and that both are necessary for estrogen production[15]. Recent tissue culture and transplant studies have shown that at least 2 of the ovarian cell types (granulosa + theca or interstitial) must be present in order for estrogen secretion to occur[16]. Thus, steroid production in the ovary is dependent on an interplay between the 2 cell types. The theca and interstitial cells are capable of producing copious amounts of androgenic steroids such as androstenedione and testosterone; these steroids are the immediate precursors of estrogens[17]. It is suggestive, from the present study, that the theca and/or interstitial cells are the major HCG receptor cell in the intact, nonpregnant rodent ovary and ovarian tumor (theca). Perhaps HCG stimulates the theca cells to produce the androgenic precursors which then interact with granulosal cells for the production of estrogens. In the corpus luteum, however, the granulosa cells are transformed into a lutein cell capable of both progesterone and estrogen biosynthesis. The lutein cells become highly sensitive to HCG since they are more capable of producing the androgenic steroid precursors due to the change in the biosynthetic pathway.

Zusammenfassung. Bei Mäusen mit Granulosa- oder Theca-Tumoren wurde die Bindung von ^{125}I-HCG untersucht. Im Vergleich zur Bindung an andere Gewebe derselben Tiere konnte eine zellspezifische Bindung im Bereich der Tumoren festgestellt werden. Die Befunde werden im Zusammenhang mit der Zweizelltheorie der ovariellen Steroidgenese diskutiert.

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N^{α}-Carbamoyl-2-*O*-Methyltyrosine-oxytocin and 1-6 α Deamino Cystathionine-2-*O*-methyltyrosine Oxytocin: Two Antagonists of Oxytocin on Amphibian Epithelial Cell Receptors

Several structural analogues of neurohypophyseal hormones have been shown to antagonize the effects of the active peptides on their different target organs (for review, see[1]). In the present study, we describe the inhibition of the hydroosmotic and natriferic effects of oxytocin on the frog skin and bladder by N^{α}-Carbamoyl-20-methyltyrosine-oxytocin: CbmOT[2] and 1–6α deaminocystathionine-2-*O*-methyltyrosine-oxytocin: MeDCOT-1[3]; the structure of these analogues[4] is described in Table 1.

Experimental. The hydroosmotic effect (increase in the net water flow along an osmotic gradient) was measured on the isolated frog bladder (*Rana esculenta*) using a previously described technique[5]. The dose-response relationship for synthetic oxytocin (Syntocinon Sandoz) was first determined using the cumulative doses technique. The affinity of oxytocin for its receptor was measured according to EGGENA[6] et al. by the PD_2 value (negative logarithm of the molar concentration of hormone in the medium (A50) yielding half the maximum response). Its apparent intrinsic activity was measured by the magnitude of the maximum biological response. After washing out the hormone and complete reversal of the hydroosmotic response, the dose-response relationship for oxytocin was again determined in the presence of a known concentration (B) of the inhibitor. The new A 50 value (A 50 B) was used to calculate the affinity constant of the inhibitors:

$$pA2 = -\log (B/(A50B/A50\text{-}1))$$

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The natriferic effect (stimulation of active sodium transport) was measured on the isolated skin by the increase in the current (SCC) flowing through the short-circuited preparation[7]. The currents flowing through 6 independent areas (0.8 cm^2 each) of the same skin were simultaneously recorded, using a previously described device[8]. The general procedure for the determination of affinity constants and intrinsic activities is similar to that described above, except that the dose-response relationships in the presence and absence of inhibitor were determined on symmetrical areas of the same preparation.

Results. High doses of CbmOT and MeDCOT-1 (final concentrations in the medium up to $2 \times 10^{-5} M$) were completely devoid of both natriferic and hydroosmotic activities but significantly shifted the dose-response relationships for oxytocin towards the highest concentrations. On the other hand, the apparent intrinsic activity of the oxytocin was not modified (Table II). This last observation suggested that CbmOT and MeDCOT-1 act as truly competitive inhibitors of oxytocin at the level of its receptor sites. The mean *pA2* values (Table I) indicate that MeDCOT-1 has a higher affinity than CbmOT. The hypothesis of there being competition at the level of the receptor sites was confirmed by the observation that these two analogues were to some extent able to stimulate the adenylcyclase system of frog skin and bladder epithelial cells in the same way as biologically active neurohypophysial peptides[9]. Thus, when the phosphodiesterase activity was inhibited with theophylline sufficiently to raise the intracellular cyclic AMP concentration to a level giving less than the maximum increase in water permeability and active sodium transport, MeDCOT-1 produced a significant biological effect (Table III). The potentiation of the theophylline effects by CbmOT was only apparent in the natriferic effect (Table I).

Discussion. Table IV gives the affinities and intrinsic activities of the two analogues studied and of their parent compounds: (*O*-methyltyrosine-oxytocin (MeOT) and 1–6 α deamino-cystathionine-oxytocin (DCOT-1). Both are expressed as a percentage of the affinity and intrinsic activity of oxytocin used as a standard. In the hydroosmotic and natriferic tests, methylation of the tyrosyl residue strongly reduced the affinity for the receptor as compared to oxytocin[10], but was compatible with the maintenance of high intrinsic activity. On the other hand, when DCOT-1 and MeDCOT-1 are compared, it may be noted that methylation of the tyrosyl residue does not lead to such a large drop in affinity, suggesting that steric modifications induced by methylation are in some way related to the presence of a sulphur atom in position 1.

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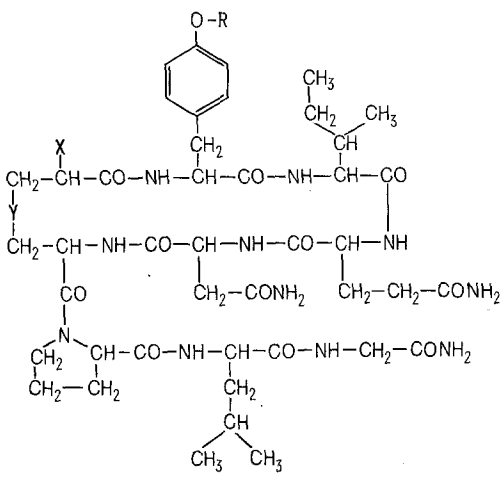

Table I. Structure of oxytocin antagonists tested

Oxytocin : Y= –S–S–
X= $–NH_2$
R= –H

CbmOT : Y= –S–S–
X= $NH_2CONH–$
R= CH_3

MeDCOT-1: Y= $–CH_2–S–$
X= H–
R= $CH_3–$

Table II. Competitive inhibition of hydroosmotic and natriferic activities of oxytocin by CbmOT and MeDCOT-1

Natriferic test	pD2	pA2	Maximum effect ($\mu A/cm^{-2}$) Without inhibitor (A)	With inhibitor (B)	A-B
CbmOT (6)	8.6±0.4[a]	4.9±0.1	17.6±5.0	14.0±6.6	3.6±4.2 NS[b]
MeDCOT-1 (5)	8.4±0.1	6.0±0.1	19.8±8.6	17.2±7.8	2.6±1.1 NS
Hydroosmotic test	*pD2*	*pA2*	Maximum effect ($\mu l/min^{-1}/cm^{-2}$) Without inhibitor (A)	With inhibitor (B)	A-B
CbmOT (5)	8.5±0.4	5.7±0.7	2.4±0.4	2.0±0.5	0.4±0.3 NS
MeDCOT-1 (10)	8.1±0.3	6.1±0.4	2.1±0.3	1.9±0.3	0.3±0.3 NS

[a] Values are means ± S.D. The number of determinations is indicated in brackets. [b] The mean values of the differences A-B were compared to 0 using the Student's *t*-test. NS, non significant at the probability level of 0.05.

Table III. Potentiation by theophylline of the effects of CbmOT and MeDCOT-1
Natriferic effect (Δ SCC: $\mu A/cm^{-2}$)

Experiment	Oxytocin	Theophylline	Theophylline + CbmOT
1	29	9.5	11
2	20	11.5	15
3	26	8.5	14.5
	Oxytocin	Theophylline	Theophylline + MeDCOT-1
4	21	9.5	19.5
5	21	13.5	19.0

Hydroosmotic effect (Δ Water flow: $\mu l/min^{-1}/cm^{-2}$)

Experiment	Oxytocin	Theophylline	Theophylline + CbmOT
6	5.8	1.3	1.1
7	6.1	0.6	0.4[a]
	Oxytocin	Theophylline	Theophylline + MeDCOT-1
8	3.5	0.2	1.9
9	3.5	0.4	1.8
10	2.0	0.5	1.2
11	2.0	0.3	1.3

The effect of a maximum dose of oxytocin was measured in both tests (Column 2). After testing the absence of response to CbmOT or MeDCOT 1 (the concentrations used were 2–3 times those giving half saturation of the receptors), the effects of an infra-maximum concentration of theophylline were measured (Column 3). At the maximum response, the same doses of CbmOT or MeDCOT-1 were added to the incubation medium. The magnitude of the response aroused is indicated in Column 4. [a] In this experiment, further addition of oxytocin led to a nearly maximum effect.

Table IV. Comparison of the affinity and intrinsic activity of CbmOT and MeDCOT-1 with those of their parent compounds

Peptide	Affinity (% of oxytocin affinity)		Intrinsic activity (% of oxytocin activity)	
	Natriferic test	Hydroosmotic test	Natriferic test	Hydroosmotic test
Oxytocin	100	100	100	100
MeOT	1.9	3.9	100[a]	90[b]
CbmOT	0.012	0.11	0	0
DCOT-1	3.4	2.1	103[c]	49[c]
MeDCOT-1	0.36	1.1	0	0

[a] Value quoted from MOREL and BASTIDE[13]. [b] Value quoted from JARD et al.[12]. [c] Value quoted from BARTH et al.[14].

Furthermore, carbamylation, or the replacing of one S atom by a methylene group, enhances the reduction in intrinsic activity induced by methylation, as is apparent in the hydroosmotic test[6,11,12]. Preliminary experiments with N^{α}-acetyl-2-*O*-methyltyrosine-oxytocin indicated that acetylation produces the same effect. However, the results obtained with this analogue were highly variable; sometimes it was completely inactive, while in other experiments it behaved like a partial oxytocin agonist.

Finally, the loss in affinity associated with all the substitution studied is more pronounced in the natriferic than the hydroosmotic test, especially for CbmOT. In connection with this observation, it is interesting to note that MeDCOT-1 was found to be active on uterotonic and antidiuretic tests[15].

Résumé. La N_{α}-Carbamoyl-2-*O*-Methyltyrosine ocytocine et la 1–6 α déaminocystathionine 2-*O*-Methyltyrosine ocytocine inhibent les effets hydroosmotique (augmentation de la perméabilité osmotique à l'eau de la vessie de Grenouille) et natriférique (stimulation du transport actif de sodium par la peau) de l'ocytocine. L'inhibition observée est de nature compétitive. Les affinités respectives de ces deux analogues, exprimées en % de l'affinité de l'ocytocine, sont de 0,012 et 0,36 sur le test natriférique et de 0,11 et 1,1 sur le test hydroosmotique.

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