In contrast, the oxytocic activities of [8-formyllysine]oxytocin are retained at a level equal to those of AVT, and the same activities of [8-citrulline]oxytocin are actually enhanced, giving rise to a striking differentiation of oxytocic and pressor-antidiuretic properties for both analogs.

A change in the stereochemistry of the arginine residue in position 8 of arginine vasotocin from an L to a D configuration results in a substantial increase in the antidiuretic activity while essentially eliminating the pressor activity. This differentiation of activities with [8-D-arginine]vasotocin is considerably more dramatic than previously observed with [8-D-arginine]vasopressin[11], [8-D-lysine]vasopressin[11] and other vasopressin analogs with an amino acid residue of D configuration in position 8[12–14]. These results have all been interpreted to indicate a more stringent requirement for a complementary charge interaction at the pressor receptor, compared to the antidiuretic receptor[7].

In the series of analogs with 3-position modifications, the N-terminal amino group is deleted. In general, deamino oxytocin compounds show increased uterotonic activity (e.g.[15]) and deamino vasopressins exhibit a high ratio of antidiuretic-to-pressor activities (e.g.[16]). The substitution of norleucine for isoleucine in [1-deamino, 3-norleucine]arginine vasotocin results in relatively high antidiuretic activity with retention of significant oxytocic activities. The replacement of isoleucine by valine is accompanied by a large reduction of all tested activities, and proline is an even less compatible replacement for the isoleucine residue. Provided that only amino acid residues with aliphatic side chains are considered as substitutions in position 3 it is likely that the decreases in potency reflect differences in binding of the analog to receptor[17], although dose response studies in appropriate in vitro assay systems are required to ascertain this point.

In conclusion, modifications of the side chains of 'corner' residues of the proposed β-turns of oxytocin – in this case residues in positions 3 and 8 – can selectively affect biological activities of the neurohypophyseal hormone analogs, although by the influence these modified side chains may have on the 'catalytic center' of the proposed 'biologically active' conformation of oxytocin[4,7] they may exert synergistic rather than strictly additive effects.

Zusammenfassung. Nachweis, dass die Substitution in Positionen 3 und 8 im Arginin Vasotocin ([8-Arginin]-Oxytocin) selektive Veränderungen verschiedener biologischer Aktivitäten hervorruft, was am Modell der Oxytocinkonformation diskutiert wird.

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[11] M. ZAORAL, J. KOLC and F. ŠORM, Collect. Czech. chem. Commun. *32*, 1242 (1967).
[12] M. ZAORAL, J. KOLC and F. ŠORM, Collect. Czech. chem. Commun. *35*, 1716 (1970).
[13] M. ZAORAL and M. FLEGEL, Collect. Czech. chem. Commun. *37*, 3350 (1972).
[14] M. ZAORAL and F. ŠORM, Collect. Czech. chem. Commun. *31*, 310 (1966).
[15] D. B. HOPE, V. V. S. MURTI and V. DU VIGNEAUD, J. biol. Chem. *237*, 1563 (1962).
[16] R. D. KIMBROUGH, JR., W. D. CASH, L. A. BRANDA, W. Y. CHAN and V. DU VIGNEAUD, J. biol. Chem. *238*, 1411 (1963).
[17] J. RUDINGER, V. PLIŠKA and I. KREJČÍ, Rec. Progr. Horm. Res. *28*, 131 (1972).
[18] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[19] R. A. MUNSICK, Endocrinology *66*, 457 (1960).
[20] R. A. MUNSICK, W. H. SAWYER and H. B. VAN DYKE, Endocrinology *66*, 860 (1960).
[21] J. M. COON, Archs int. Pharmacodyn. *62*, 79 (1939).
[22] *The Pharmacopeia of the United States*, 17th Rev. (Mack Printing Co., Easton, Pa. 1965), p. 749.
[23] W. A. JEFFERS, J. J. LIVEZEY and J. H. AUSTIN, Proc. Soc. exp. Biol. Med. *50*, 184 (1942).
[24] R. L. HUGUENIN and R. A. BOISSONNAS, Helv. chim. Acta *43*, 182 (1962).
[25] Acknowledgment. We thank Dr. KLAUS LÜBKE, Schering AG, West Germany, for generously supplying us with the hormone analogs.

Hyperglucagonemia, Hypocalcemia and Diminished Gastric Blood Flow-Evidence for an Etiological Role in Stress Ulcer of Rat

In the past many pathophysiological aspects of stress induced ulcers in man and experimental animals have been studied intensively. At present, despite increased knowledge of the role which various factors possibly involved might play, such as histamine and the magnitude of gastric mucosal perfusion, this disorder is far from being fully understood.

Pancreatic glucagon (pGl) is known to suppress both gastric acid secretion[1,2] and blood perfusion[3]. In addition to hypoglycemia and amino acids infusion[4], this hormone is released from the pancreas by stress[5]. Hypothesizing that pGl might be a important substance during the events finally leading to mucosal lesions and ulcers, we studied several groups of rats using local oxygen pressure (pO_2) in gastric mucosa as a reliable index for blood perfusion (described in detail elsewhere[6]) and its dependency upon pGl concentration. In order to clarify whether pGl also may be functionally related to actual serum calcium and gastrin, these parameters were measured in intact and adrenalectomized rats.

Materials. 3 groups of male SPF Wistar rats, approx. 200 g body wt. (Mus Rattus GmbH., Brunnthal/Germany) were randomly divided into intact, sham-operated (laparotomy only) and adrenalectomized animals, 1 moiety serving as control. The other half was stressed by the restraint technique[7] at room temperature for 24 h. Food

[1] T. M. LIN and G. F. SPRAY, Archs int. Pharmacodyn. *191*, 88 (1971).
[2] E. KOKAS, S. H. KAUFMAN and J. C. LONG, Z. vergl. Physiol. *74*, 315 (1971).
[3] T. M. LIN and M. W. WARRICK, Gastroenterology *61*, 328 (1971).
[4] A. OHNEDA, M. SATO and K. MATSUDA, Tohoku J. exp. Med. *107*, 241 (1972).
[5] S. R. BLOOM, P. M. DANIEL and D. J. JOHNSTON, Q. Jl. exp. Physiol. *58*, 99 (1973).
[6] W. SCHELLERER, P. O. SCHWILLE and P. HERMANEK, Langenbecks Arch. Chir., Suppl. Chir. Forum, in press (1974).
[7] D. A. BRODIE and H. M. HANSON, Gastroenterology *38*, 353 (1960).

Ulcer index (UI), oxygen partial pressure (pO_2), immunoassayable Glucagon (pGl) and Gastrin (G), total serum calcium (Ca_t) and protein (TP) in rats without (C) and following stress (S)

Groups	Stress h	U.I.	pO_2 mm Hg	G pg/ml	pGl pg/ml	Ca_t mg/100 ml	TP g/100 ml
1. Intact (C)	24	0	19.08	137	135	10.49	6.74
		(19)	0.34	17	13	0.14	0.18
			(16)	(18)	(20)	(19)	(14)
2. Intact (S)	24	20.75	9.77	119	333	8.60	6.13
		(18)	0.40	12	67	0.20	0.22
			(19)	(18)	(19)	(20)	(13)
3. Sham-operated (C)[d]	24	0	18.58	162	170	10.37	6.51
		(17)	0.27	13	19	0.11	0.18
			(19)	(18)	(19)	(19)	(19)
4. Sham-operated (S)[d]	24	19.85	9.92	96	506[a]	8.71	6.04
		(18)	0.20	5	79	0.15	0.17
			(20)	(20)	(19)	(20)	(14)
5. Adrenalectomized (C)[d]	6–8	8.03	15.42[c]	405[c]	284[c]	10.86	6.58
		(17)	0.37	56	41	0.12	0.33
			(22)	(18)	(17)	(16)	(10)
6. Adrenalectomized (S)[d]	6–8	13.70	8.55[a]	165	678[b]	10.31[c]	5.94
		(19)	0.32)	20	95	0.17	0.22
			(19)	(13)	(91)	(11)	(5)

Mean ± SEM. (), Number of animals. [a] $p < 0.05$; [b] < 0.01; [c] < 0.001 (Student's t-test: 3 vs 1; 5 vs 1; 4 vs 2; 6 vs 2. [d] Laparotomy only and adrenalectomy 10 days before experiments.

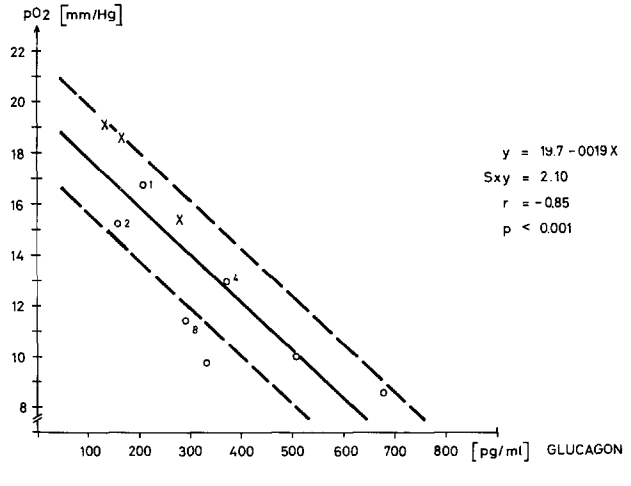
(normal laboratory diet, Altromin GmbH., Lage/Germany) and drinking water were withheld 24 h prior to the experiments (controls). After stress procedure, all animals were anesthetized by pentobarbital-Na, fixed on a heated (37 °C) operating table and laparotomized in order to expose gastric surface and allow bleeding from the aorta.

Methods. Determination of pO_2 was done by polarography, applying a 25 µm platinum electrode (Transidyne General Corp., Michigan/USA). Final individual values were established by recording 24 single values out of 4 topographically fixed regions within the glandular stomach on a Servogor recorder, and calculation of mean values.

Measurement of pGl and G were done by radioimmunoassay[8]. The former was set up using the highly specific antibody 30 K[9], the latter by a commercially available kit (Isotopendienst West GmbH., Frankfurt/Germany) and Human Gastrin I as standard. Separation of bound antibody from free hormone by addition of 0.5 ml/tube charcoal dextran mixture[10]. Intra and inter assay coefficient of variation were 9.0 and 13.2 (pGl), 8.4 and 12.5 (G) respectively. Serum Ca was determined by EDTA-titration using Calcein as indicator (Marius, Kipp & Zoonen, Schönberg/Germany); total protein, using biuret reagent.

Results and discussion. (Table) 1. Stress induced mucosal lesions. Although in this stress model formation of classical ulcers occurs in a few cases only, and tends to result in mucosal lesions of varying steepness, quantitative histological examination (unpublished) demonstrates that the ulcer index (UI), according to Brodie and Hanson[7], can be accepted as a reliable criterium of gastric lesions due to stress. In intact and sham-operated rats, zero UI is associated with nearly equal pO_2 (19.08 vs 18.58). Both stress alone and adrenalectomy alone severely increase UI and reduce gastric perfusion, the nadir of pO_2 being observed in adrenalectomized rats following stress.

2. Glucagon, gastrin and calcium. Compared with control groups, pGl in stressed animals was definitely elevated. This result is in accordance with the recent view that catecholamines are potent stimuli to the α-cells[11,12]. Moreover, the finding that highest pGl occurs in adrenalectomized rats during stress would be consistent with the assumption that extra-adrenal sources of catecholamines might contribute to release of pGl. Regression analysis of perfusion data favours the aforementioned



Relationship between plasma glucagon and gastric perfusion as indicated by pO_2 in stressed rats (○) and controls (X). Numbers denote duration of stress in h in 4 groups of animals not delineated in this report.

[8] E. Aguilar-Parada, A. M. Eisentraut and R. H. Unger, Am. J. med. Sci. *257*, 415 (1969).
[9] Supplied by Dr. R. H. Unger, Dallas, USA.
[10] V. Herbert, K.-S. Lau and C. W. Gottlieb, J. clin. Endocr. Metab. *25*, 1375 (1965).
[11] J. Iversen, J. clin. Invest. *52*, 2102 (1973).
[12] N. J. A. Vaughan, S. R. Bloom, O. Ogawa, P. M. Bircham and A. V. Edwards, Experientia *29*, 805 (1973).

hypothesis that pGl would indeed be involved in depression of gastric blood flow (Figure).

Immunoassayable G, on the other hand, tends to decline during stress (control vs stress, Table) but increases markedly after adrenalectomy. In the light of greater sensitivity of adrenalectomized rats to ulcerogenic factors, it would be of interest to discover whether there is a feed-back mechanism not yet described between gastrin(s) and substances derived from the adrenals. As indicated by serum calcium in the intact and sham-operated groups during stress, it seems to us that a hypocalcemic principle is likely to be stimulated from normal adrenals to enter general circulation and suppresse activity of gastrin(s) production sites[13]. Although earlier work by KAPLAN et al.[14,15] suggested a role for the adrenals in calcium homeostasis, our present report is, we believe the first to deal with a functionally related trias of findings leading to gastric ulcers originally induced by stress situations: hyperglucagonemia, hypocalcemia and reduced gastric mucosal blood supply.

Further experiments are under way to elucidate the pathophysiological validity of each single parameter, with special respect to actual plasma gastrin(s). In addition, they may help to find out what kind of therapy should be envisaged in the presence of stress ulcer in humans.

Zusammenfassung. Unter Zwangshaltung entwickeln intakte, scheinoperierte und adrenalektomierte Ratten eine Drosselung der Magenperfusion und unterschiedlich häufig Nekrosen und Erosionen in der Magenmukosa. Dieses Phänomen ist von einem erheblichen Anstieg an Pankreasglucagon begleitet, während Gastrin unter Stress eher abfällt.

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12 February 1974.

[13] H. D. BECKER, S. J. KONTUREK and D. D. REEDER, Am. J. Physiol. *225*, 277 (1973).
[14] E. L. KAPLAN, G. W. PESKIN and C. D. ARNAUD, Surgery *66*, 167 (1969).
[15] E. L. KAPLAN, C. D. ARNAUD and B. J. HILL, Surgery *68*, 146 (1970).
[16] Acknowledgment. We are gratefully indebted to K. SCHWILLE, A. WELLMANN and G. BESOLD for skillful technical assistance.

Action of L-Dopa on the Gonads of Male Rats

Melatonin (5-methoxy-N-acetyltriptamine) is a hormone secreted by the pineal gland (LERNER[1]), which has a decolorant action on the skin of amphibians[1], an antigonadotrophic action (WURTMAN et al.[2]), a hypothermal action (BARCHAS et al.[3]), and a hypnotic action (MARCZYNSKI[4]). It has been shown that L-Dopa produces an increase of melatonin secretion by the pineal gland (WURTMAN et al.[5]). In this paper, we have tried to study the effect of the administration of L-Dopa on the gonads of male rats.

Materials and methods. 39 male rats, Wistar strain, 200–250 g, were used; 300 mg/kg of L-Dopa hydrochloride were administered s.c. to a group of 20 rats, 3 times a week for 1 month. Another group of 19 rats was used as control; these rats received distilled water on the same days and by the same route. After 30 days, the rats were killed and their testicles, seminal vesicles and prostate removed. The calculation of results was made using Student's *t*-test.

Results. The administration of L-Dopa to male rats diminished the weight of testicles, seminal vesicles and prostate (Table).

Action of L-Dopa on the weight of sexual organs of male rats

Treatment	Testicles (each)	Prostate	Seminal vesicles[a]
Control	1637.73 ± 40.825	136.105 ± 10.55	281 ± 17.431
L-Dopa	1418.6 ± 43.973 ($p < 0.001$)	92.2 ± 5.826 ($p < 0.005$)	229.3 ± 12.276 ($p < 0.01$)

[a] Mean values in mg ± S.E.M. The figures in brackets show the statistical significance.

Discussion. WURTMAN et al.[2,5] demonstrated the inhibitory action of melatonin on the growth and weight of sexual organs of rats and hamsters. RABADÁN[6] has also observed an inhibitory action of melatonin on the weight of prostate, seminal vesicles and ovaries of rats. The results obtained appear to be due to the action of L-Dopa on the pineal gland, as WURTMAN et al.[7] have demonstrated that s.c. administration of 300 mg/kg of L-Dopa to rats produces an increase of the melatonin contents in the pineal gland. The increase of level of melatonin would be responsible of the decreased weight of testicles, seminal vesicles and prostates.

Resumen. La administración de 300 mg/kg L-Dopa, a ratas machos, por vía subcutánea, tres días a la semana durante un mes, produce una disminución significativa – del peso de testículos, vesículas seminales y próstata.

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[1] A. B. LERNER, J. D. CASE, Y. TAKAHASHI, T. H. LEE and W. MORI, J. Am. chem. Soc. *80*, 2587 (1958).
[2] R. J. WURTMAN, J. AXELROD and E. W. CHU, Science, *141*, 277 (1963).
[3] J. BARCHAS, F. DA COSTA and S. SPECTOR, Nature, Lond. *214*, 919 (1967).
[4] T. I. MARCZYNSKI, N. YAMAGUCHI, G. H. LING and L. GLODZINSKA, Experientia *20*, 435 (1964).
[5] R. J. WURTMAN, J. AXELROD and L. PHILLIPS, Science *142*, 1071 (1963).
[6] F. P. RABADÁN, Arch. Fac. Med. Madrid, *23*, 295 (1973).
[7] H. J. LYNCH, P. WANG, R. J. WURTMAN, Life Sci. *12*, 145 (1973).