Uperolein was demonstrated to occur also in the 60% ethanol eluate obtained from an alumina column loaded with an extract of the skin of *Uperoleia marmorata* (130 adult specimens = 8 g dried skin).

In addition to uperolein, *Uperoleia marmorata* skin contained another physalaemin-like peptide, closely related to, but not identical with, uperolein, which emerged mainly in the 30% ethanol eluate. On high voltage electrophoresis, it showed the same electrical mobility as physalaemin ($E_{1\cdot8}$ = 0.43 Glu; no mobility at pH 5.8).

A physalaemin-like peptide different from uperolein also appeared in 40 and 30% ethanol eluates of the *U. rugosa* extract, but it is not known whether this second peptide is identical with the second peptide found in the 30% ethanol eluate of *U. marmorata*. Elucidation of the structures of these peptides is in progress.

Riassunto. Gli estratti metanolici di pelle degli anfibi australiani *Uperoleia rugosa* e *Uperoleia marmorata* contengono due o tre polipeptidi fisaleminosimili. Il più importante di essi, l'uperoleina, è stato isolato allo stato di purezza e all'analisi sequenziale è risultato essere l'endecapeptide Pro^2-Ala^6-fisalemina.

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Inhibition of Catecholamine and 5-Hydroxytryptamine Induced Enzyme Secretion from the Guinea-Pig Submandibular Gland by 2-Bromo-D-Lysergic Acid Diethylamide[1]

5-Hydroxytryptamine (5-HT, serotonin) has recently been shown to enhance enzyme secretion from the rat and rabbit parotid glands[2,3], as well as from the guinea-pig submandibular gland[4,5]. In the latter gland, the secretory effect of 5-HT can be abolished by both α- (phenoxybenzamine) and β- (propranolol) adrenergic blocking agents in vitro[5]. Furthermore, the amylase secretory response of 5-HT, injected i.v. into rabbits, can be partly (45%) inhibited by initial treatment of the animals with propranolol[3]. The exact mode of action, however, of this biogenic monoamine, as well as the presence of a specific 5-HT receptor in mammalian salivary glands has not been established. In addition, 5-HT markedly enhances fluid secretion from the abdominal salivary gland of the blowfly, and it has been suggested that this effect is mediated via cyclic AMP[6,7].

2-bromo-D-lysergic acid diethylamide (BOL 148) is an effective 5-HT-blocker in various tissues[8–11]. In the present investigation, the effect of BOL 148 on 5-HT, dopamine, noradrenaline, adrenaline and dibutyryl cyclic AMP-theophylline-induced peroxidase and amylase secretion from the guinea-pig submandibular gland was studied in vitro.

Materials and methods. Male guinea-pigs, 3 months of age, weighing roughly 300 g, were used. The animals were starved overnight before being anesthetized by an i.p. injection of sodium pentobarbital (Mebumal®, ACO, Sweden). The submandibular glands of 2 animals were rapidly excised and extraglandular tissue removed under a stereomicroscope. The glands were cut into small fragments and randomly distributed among the incubation vessels. The basal medium used was a Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with pyruvate, glutamate and fumarate[12] and also containing albumin

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Table I. Peroxidase release. In vitro effects of BOL 148 on the secretion of peroxidase from guinea-pig submandibular gland

Secretagogues	No. of experiments	Non-stimulated secretion	Control (stimulated secretion)	Test Secretagogue + BOL 148 (2×10^{-4} *M*)	Difference (test-control)
DBcAMP (1 m*M*) + theophylline (5 m*M*)	5	1.6 ± 0.14	10.7 ± 3.28	14.8 ± 4.07	+ 4.1 ± 3.80
Noradrenaline (10^{-5} *M*)	5	1.1 ± 0.14	14.9 ± 3.10	0.84 ± 0.15	−14.0 ± 3.16[c]
Adrenaline (10^{-5} *M*)	5	1.3 ± 0.20	6.2 ± 1.17	0.74 ± 0.09	− 5.4 ± 1.21[c]
5-HT (10^{-4} *M*)	5	1.1 ± 0.14	9.6 ± 1.07	1.8 ± 0.35	− 7.8 ± 1.13[b]
Dopamine (10^{-4} *M*)	4	1.7 ± 0.11	17.8 ± 2.03	1.8 ± 0.46	−16.1 ± 2.17[b]

After a preincubation period of 30 min at 37 °C the specimens were incubated in a supplemented Krebs bicarbonate buffer for 60 min with listed concentrations of secretagogues. BOL 148 (2×10^{-4} *M*) was present during both the preincubation and incubation periods. The enzyme release is expressed as percentage of the total peroxidase activity in tissue and medium. Mean values (%) ± S.E.M. for indicated number of experiments. [a] $P < 0.001$; [b] $p < 0.01$; [c] $p < 0.05$.

Table II. Amylase release. In vitro effects of BOL 148 on the secretion of amylase from guinea-pig submandibular gland

Secretagogues	No. of experiments	Non-stimulated secretion	Control (stimulated secretion)	Test Secretagogue + BOL 148 (2×10^{-4} *M*)	Difference (test-control)
DBcAMP (1 m*M*) theophylline (5 m*M*)	5	14.0 ± 3.47	36.4 ± 6.08	42.2 ± 8.00	+ 5.8 ± 7.31
Noradrenaline (10^{-5} *M*)	5	6.1 ± 0.85	29.9 ± 2.16	8.2 ± 1.15	−21.7 ± 3.00[b]
Adrenaline (10^{-5} *M*)	5	16.5 ± 1.21	40.3 ± 2.58	15.8 ± 2.13	−20.4 ± 3.15[b]
5-HT (10^{-4} *M*)	5	6.1 ± 0.85	17.8 ± 2.35	6.6 ± 0.76	−11.2 ± 2.64[c]
Dopamine (10^{-4} *M*)	4	13.7 — 4.47	44.3 — 2.26	27.1 — 3.87	−17.2 — 3.43[c]

The measurements were performed on the same samples as used for the peroxidase analyses (Table I). The amylase release is expressed as percentage of the total amylase activity in tissue and medium. Mean values (%) ± S.E.M. for indicated number of experiments.[a] $P < 0.001$; [b] $p < 0.01$; [c] $p < 0.05$.

(5 mg/ml) and glucose (0.6 mg/ml). A preincubation period of 30 min preceded the incubations with various secretagogues and BOL 148 (2-bromo-D-lysergic acid diethylamide) as described in the Tables. The 5-HT antagonist was also present during the preincubation period.

Preincubation as well as incubation was carried out in 3 ml of medium at 37 °C under a continuous flow of O_2–CO_2 (95:5) as previously described[13]. After 60 min of incubation the specimens were separated from the incubation media and were homogenized in 3 ml of 50 m*M* phosphate buffer (pH 6.9) using an Ultra-Turrax homogenizer (Janke und Kunkel K.G., Staufen, Germany). The homogenates were centrifuged at $3{,}000 \times g$ for 5 min. Both incubation media and supernatants were assayed for peroxidase and amylase.

Peroxidase assay. Samples of tissue extract and incubation media were added to 16.7 m*M* pyrogallol in 200 m*M* sodium phosphate buffer (pH 6.0) in a total volume of 3.0 ml. H_2O_2 was added to a final concentration of 1.67 m*M*, and the peroxidase activity was calculated from the initial rate of increase in absorbance at 400 nm and related to the tissue wet weight. Peroxidase release was expressed as percentage of the total peroxidase activity in tissue and medium.

Amylase assay. Samples of tissue extracts and incubation media were appropriately diluted with 50 m*M* phosphate buffer (pH 6.9) and assayed for amylase using a micro-modification of the 3,5-dinitrosalicylate method with 2% soluble starch as substrate[13]. Amylase release was expressed as percentage of the total amylase activity in tissue and medium.

Statistical evaluation. The statistical probability that the effect of additives to the incubation media was due to chance, was estimated from the mean difference between test and control incubations in a series of identical but separate experiments.

Chemicals. Soluble starch, 3,5-dinitrosalicyclic acid and pyrogallol were obtained from E. Merck E.G., Darmstadt, Germany, L-noradrenaline bitartrate was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks, England. 5-Hydroxy-tryptamine creatinine sulfate (5-HT), 3-hydroxytyramine-HCl (Dopamine) and L-adrenaline bitartrate were purchased from Sigma Chemical Co., St. Louis, Mo., USA. Theophylline was from Mann Research Laboratories Inc., New York, N.Y., USA. N^6-2-*O*-dibutyryl-adenosine 3′:5′-cyclic monophosphate was obtained from C.F. Boehringer und Söhne G.m.b.H., Mannheim, Germany. The 5-HT antagonist 2-bromo-D-lysergic acid diethylamide (BOL 148) was kindly donated by Sandoz A.G., Basel, Switzerland.

Results and discussion. The release of both peroxidase and amylase was stimulated by a combination of DBcAMP and theophylline, noradrenaline, adrenaline, dopamine as well as by 5-HT (Tables I and II). All concentrations of the various amines were chosen so as to elicit a maximum secretory response[4,5,14]. The addition of BOL 148 (2-bromo-D-lysergic acid diethylamide), a 5-HT antagonist, did not affect the DBcAMP-theophylline induced enzyme release. The drug alone has no effect on basal amylase secretion (7.4 ± 2.61%; $n = 5$) as compared to simultaneous control incubations (9.9 ± 2.93%). BOL 148 totally abolished the 5-HT induced peroxidase and amylase secretion. Moreover, enzyme discharge caused by noradrenaline, adrenaline and dopamine was also blocked by this compound. BOL 148 was equally effective in antagonizing the secretory effects of the different catecholamines as in the case of the 5-HT-induced enzyme discharge.

In the present investigation, BOL 148 was used as a 5-HT antagonist in an effort to differentiate selectively between serotonin receptors and adrenergic receptors. However, BOL 148 inhibits not only enzyme secretion induced by 5-HT but also noradrenaline, dopamine and adrenaline secretory effects. On the other hand, the drug has no effect whatsoever on enzyme release provoked by dibutyryl cyclic AMP in combination with theophylline. The latter finding would seem to exclude a non-specific interference with the secretory process. Also in other secretory cell systems (e.g. the pancreatic betacell) a proposed specific serotonin antagonist, methysergide maleate, abolishes not only the effect of 5-HT but also that of noradrenaline and dopamine[15,16]. Moreover, both α- and β-adrenergic blocking agents inhibit 5-HT-induced enzyme secretion from submandibular gland[5], and taken together these findings might indicate a close relationship between the postulated receptor of 5-HT and that of the catecholamines. In conclusion, our results indicate that BOL 148 cannot be considered a specific 5-HT antagonist in salivary glands; whether or not a serotonin (5-HT) receptor really exists in these glands remains to be elucidated.

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Zusammenfassung. Isolierte Submandibularisdrüsen des Meerschweinchens wurden in physiologischer Pufferlösung mit sekretionsstimulierenden Substanzen inkubiert. Dopamin, Noradrenalin, Adrenalin und 5-HT induzieren die Sekretion von Peroxydase und Amylase, während der Serotoninantagonist BOL 148 (2-Bromo-D-Lysergsäure Dietylamid) eine beinahe vollständige Sekretionshemmung hervorruft. BOL 148 hat sich also nicht als spezifisch 5-HT-hemmend an der Meerschweinchenspeicheldrüse erwiesen.

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Dissociation of Erythrocyte Catalase into Subunits and Their Re-association

Catalase (E.C. 1.11.1.6.) of various origin has been shown to consist of 4 identical subunits with a molecular weight of approximately 60,000[1-3]. The dissociation of this oligomer-enzyme into dimer and monomer particles proceeds slowly when kept at neutral pH in dilute solution[4]. However, this decomposition can be accomplished within a few minutes in concentrated urea solution or by a shift of pH (below 4 or above 11,[5,6]). Under appropriate experimental conditions, this interconversion is reversible, thus permitting a study of the decomposition of the catalase molecule as well as its re-association from subunits.

In order to get more insight into the forces acting between subunits, both reactions have been followed. This is facilitated by the fact that only the catalatic activity – but not the peroxidatic activity – of this enzyme is lost after its dissociation into subunits. Furthermore, antigenic determinants specific for the tetramer particle disappear upon dissociation. This dissociation – re-association process permits a direct follow-up of alterations in both enzymatic and antigenic properties of the particles involved. The rate of re-association can be influenced by other proteins in the solution.

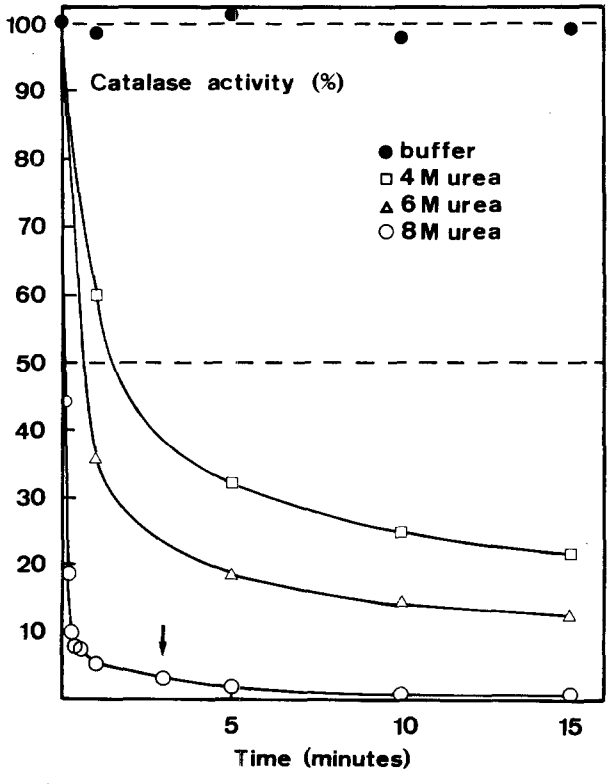

Fig. 1. Inactivation of human erythrocyte catalase in a final concentration of 4 *M* urea (□—□); 6 *M* urea (△—△); 8 *M* urea (○—○); control in 0.1 *M* phosphate buffer pH 7.2. (●—●).

Materials and methods. Human erythrocyte catalase was isolated from normal blood by the method of Mörikofer et al.[7] and Aebi et al.[4]. The effector proteins used were Bovine serum albumin, human IgM (gift from Prof. Barandun) and the IgG fractions of the anti-human erythrocyte catalase antibody[8].

Inactivation of catalase was achieved essentially by the method of Shpitsberg[6]. Samples containing 5.6 mg/ml catalase were brought to an 8, 6 and 4 *M* urea-concentration by using a 10 *M* urea solution adjusted to pH 7 with HCl. The catalase in urea was left at 20 °C for time periods from a few sec up to 15 min. Inactivation was stopped by diluting the samples with 0.1 *M* phosphate buffer pH 7.2 to a 3 *M* urea-concentration.

Re-activation studies were performed with samples containing 16.3 mg/ml catalase, which had been inactivated with urea for 3 min at 20 °C (final concentration 8 *M*). The samples were then diluted to a 3 *M* urea-concentration by the addition of 0.1 *M* phosphate buffer pH 7.2, or by the same buffer containing 10 mg/ml final concentration of various proteins. Re-activation was achieved by open dialysis of 1–2 ml samples in an ice-bath against 500 ml of the phosphate buffer for up to 8 h with a change of buffer after 6 h. The samples in the dialysis bags were mixed prior to the removal of aliquots for analysis at various time intervals.

Catalase activity was determined spectrophotometrically by direct measurement of the decrease of light absorption at 240 nm, caused by the decomposition of hydrogen peroxide by catalase[9,10]. Urea concentration was mea-

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