the bottom layer (A) of the system *n*-BuOH-EtOH-H_2O (5:1:8), NaOH was added to pH 3.2 and the solution was extracted with the top layer (B) of the same system. Evaporation of the solvent left a crude residue (XVII) that was dissolved in A and made basic with NaOH to pH 11. After 3 h HCl was added to pH 3.2 and the solution extracted with B. Evaporation of the solvent left a residue of crude peptide[7] which was eluted from DEAE-Sephadex (OH^-) with 1*M* $(NH_4)_2CO_3$ buffer[8]. A further purification was achieved by counter-current distribution in the system *n*-BuOH-EtOH-H_2O (5:1:8). After deionization on Amberlite CG 50 (H^+) the solution was evaporated in vacuo: the residue (XVIII) (m.p. 224–226° dec.; $[\alpha]_D^{20}$ − 26°, c 1, DMF; $E_{5.8}$ = 0.43 Glu; $E_{1.9}$ = 0.53 Cys (SO_3H). *Anal.* Calcd. for $C_{58}H_{73}N_{13}O_{21}S_2$: C 51.5; H 5.4; N 13.5. Found C 51.3; H 5.7; N 13.1) was found homogeneous and showed the same electrophoretic and chromatographic properties, the same behaviour towards chymotrypsin, subtilisin and the same degradative pattern and biological properties[9] of natural caerulein, thus confirming the formula deduced from degradative experiments[10,11].

Riassunto. Viene riportata la sintesi della piroglutamil-glutaminil-aspartil-tirosil(O-solfato)-treonil-glicil-triptofanil-metionil-aspartil-fenilalaninamide, un peptide identico per proprietà chimiche, fisiche e biologiche alla caeruleina.

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[7] Electrophoretic analysis reveals that the residue contains caerulein, de-sulphated caerulein and polisulphated caerulein (probably mixed anhydrides aspartic acid $-SO_3$).

[8] We are indebted to Dr. A. ANASTASI for these purification procedures.

[9] We are indebted to Prof. V. ERSPAMER for these assays.

[10] We wish to thank Dr. B. CAMERINO, Director of this Research Institute, for his interest in this work.

[11] The synthesis of a number of fragments and analogues of caerulein is currently under way.

Pharmacological Actions of Caerulein[1]

The dacapeptide caerulein (natural caerulein from *Hyla caerulea* skin[2] and synthetic caerulein[3]) displayed a number of pharmacological actions on vascular and extravascular smooth muscles as well as on some external secretions.

Action on systemic blood pressure. The i.v. injection of caerulein caused in the dog a pressure fall which was satisfactorily proportional to the dose, especially in its duration. It lasted considerably longer than that caused by bradykinin or physalaemin. Tachyphylaxis was either lacking or moderate. The threshold i.v. dose of caerulein ranged between 0.01 and 0.1 μg/kg, but even doses of 100–1000 μg/kg could be tolerated and recovery was complete. The threshold dose by i.v. infusion was 5–20 ng/kg/min. Considerably larger doses were required by s.c. route.

The polypeptide lowered the blood pressure also in humans and in the rabbit. In the cat its action was erratic, less intense and there was often tachyphylaxis; in the rat it generally caused a hypertensive or biphasic response.

By intradermal injection into the human forearm caerulein increased the capillary permeability and caused a reaction which was approximately half as intense as that caused by bradykinin. The polypeptide, however, was 5000 times less active than bradykinin on the permeability of the skin capillaries of the guinea-pig.

Action on extravascular smooth muscle. Caerulein generally displayed a poor stimulant action on isolated preparations of intestinal and uterine smooth muscle. The most important exception was the isolated gall bladder. That of the guinea-pig was contracted by concentrations of caerulein as low as 1 ng/ml, that of the rabbit and the sheep by concentrations as low as 0.2 ng/ml and 0.1 ng/ml, respectively.

The in situ gall bladder of the guinea-pig was tremendously sensitive to caerulein, the i.v. threshold dose being 0.2–0.6 ng/kg. There was an excellent dose/response relationship and there was no sign of tachyphylaxis, even over long periods of time. One μg caerulein was equiactive to 40–50 Ivy dog units of cholecystokinin, i.e. to 7–15 μg of pure cholecystokinin-pancreozymin[4].

Like that of the guinea-pig, the in situ gall bladder of the dog was potently contracted by caerulein, the threshold dose being apparently of the order of a few ng/kg. In normal humans, caerulein produced a contraction of the gall bladder at i.v. doses as low as 1–2 ng/kg (BRAIBANTI et al., personal communication).

Another aspect of the stimulant action of caerulein on the in situ gastrointestinal smooth muscle was represented by emesis and diarrhoea as seen in the intact conscious dog after administration of the polypeptide. The threshold emetic dose was approximately 0.5 μg and 2 μg/kg, by i.v. and s.c. route, respectively.

In an anaesthetized dog provided with a denervated fundic pouch, caerulein produced contraction of the gastric musculature at i.v. doses as low as 2–6 ng/kg.

Action on secretions associated with the digestive tract. (a) *Gastric secretion.* In conscious dogs provided with denervated fundic pouches, the s.c. injection of caerulein stimulated both the acid flow and the volume of gastric juice. The threshold dose was 0.1–0.5 μg/kg and the magnitude of the responses was proportional to the dose administered. The effects produced by a single s.c. dose

[1] Supported by grants from the Consiglio Nazionale delle Ricerche, Roma.

[2] A. ANASTASI, V. ERSPAMER and R. ENDEAN, Experientia *23*, 699 (1967).

[3] L. BERNARDI, G. BOSISIO, R. DE CASTIGLIONE and O. GOFFREDO, Experientia *23*, 700 (1967).

[4] A generous sample of synthetic human gastrin-I was kindly set at our disposal by Dr. R. C. SHEPPARD, The Robert Robinson Laboratories, University of Liverpool, and generous samples of pure secretin and of pure cholecystokinin-pancreozymin by Prof. E. JORPES, Department of Chemistry, Karolinska Institutet, Stockholm.

lasted 90–150 min. As well as the effects mentioned, the s.c. injection of 0.5–1 μg/kg of caerulein caused a large output of pepsin.

Because of the close structural similarity between the *H. caerulea* peptide and gastrin, the effect of caerulein on gastric secretion of the dog was not unexpected. On a weight basis, caerulein was approximately 3 times as active as human gastrin-I[4].

In the perfused stomach preparation of the rat, caerulein produced a conspicuous increase in the total acid output measured over 20 min periods. The threshold dose was 15–25 ng/kg by the i.v. route and approximately 5 μg/kg by the s.c. route. For i.v. doses ranging between 20 and 200 ng/kg there was a good dose/effect relationship.

If, during the i.v. infusion of histamine at a rate itself ineffective or poorly effective on acid flow, caerulein is injected i.v., a conspicuous potentiation of the magnitude and an even greater potentiation of the duration of the secretory response to the polypeptide were observed. The histamine liberator compound 48/80 strongly reduced acid flow whilst aminoguanidine, an inhibitor of diamine-oxidase, increased it markedly.

On a weight basis, caerulein was 10–50 times as potent as human gastrin-I and 15–20 times as potent as carbachol.

Similar results were obtained when the rat stomach with ligated pylorus was used.

Preliminary experiments revealed that caerulein conspicuously increased the active transport of chloride by the isolated gastric mucosa of *Rana esculenta*. The threshold concentration was of the order of 0.003–0.01 ng/ml. On a weight basis, human gastrin-I was at least 300–1000 times less active (PESENTE et al., to be published).

(b) *Pancreatic secretion.* The administration of caerulein to anaesthetized dogs with the main pancreatic duct cannulated resulted, in each case, in a prompt increase in the volume of pancreatic juice. Unlike the juice produced by secretin but like that produced by pancreozymin, the juice produced by caerulein was rich in enzymes (amylase) and dry residue. The threshold dose was 3–6 ng/kg by rapid i.v. injection, 0.3–0.6 ng/kg/min by i.v. infusion and 100 ng/kg by the s.c. route. The magnitude of the response was directly related to the dose administered, and even at shock levels of blood pressure stimulation of pancreatic secretion could be observed.

If increase in volume flow only was considered, 1 μg caerulein was equiactive to 35–40 μg human gastrin-I, 1–3 Jorpes clinical units of secretin or 10–20 μg pure cholecystokinin-pancreozymin.

Results similar to those described above for anaesthetized dogs were obtained if anaesthetized cats were used.

(c) *Biliary secretion.* In anaesthetized dogs caerulein elicited a powerful contraction of the gall bladder and apparently increased the flow of hepatic bile.

An evident increase in the rate of biliary flow occurred in the anaesthetized rat after administration of caerulein. The threshold i.v. dose was 1 μg/kg. Single doses of 2–5 μg/kg caused, over an approximately 2 h period, a 20–30% increase in the volume of bile produced. The dry residue content and cholesterol content of caerulein-bile was as high, or higher than the dry residue and cholesterol content of control-bile. With repeated doses the effect was more intense and could be sustained for 4–6 h.

On a weight basis, human gastrin-I showed 2% of the activity of caerulein.

From the above data, it can be seen that the relatively small molecule of caerulein possesses an astonishingly versatile and powerful pharmacological activity. At the same time, it mimics many of the effects of bradykinin, gastrin and cholecystokinin-pancreozymin.

The study of one natural and several synthetic caerulein-like polypeptides is in progress.

Full reports and discussions of the experiments and results described in this paper will be published elsewhere.

Riassunto. La caeruleina, nonapeptide attivo della pelle di *Hyla caerulea*, possiede un insieme di potenti azioni farmacologiche sulla muscolatura liscia vasale ed extravasale e sulle secrezioni del tubo digerente. La caeruleina provoca una relativamente prolungata caduta della pressione del sangue nel cane e nel coniglio, contrae potentemente la muscolatura in situ dello stomaco, dell'intestino e soprattutto della cistifellea, stimola poderosamente la secrezione gastrica, la secrezione pancreatica e, in misura minore, la secrezione biliare. In queste sue multiformi azioni farmacologiche la caeruleina risulta più potente rispettivamente della bradichinina, della gastrina e della colecistochinina-pancreozimina.

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Isolation and Structure of 2,4-Dihydroxy-3,5,6-Trimethylbenzoic Acid from *Mortierella ramanniana*

We have isolated a highly substituted 6-methylsalicylic acid derivative from the fungus *Mortierella ramanniana* var. *angulispora* (Naumov) Linnemann and have determined its structure to be 2,4-dihydroxy-3,5,6-trimethylbenzoic acid (I). The fungus was grown with agitation and aeration for 5 days in a medium composed of glucose (3%), ammonium acetate (0.2%), sodium sulfate (0.1%), potassium acid phosphate (0.075%), potassium chloride (0.03%), magnesium acetate tetrahydrate (0.01%), ferric chloride hexahydrate (0.002%) and protein hydrolyzate (0.1%). The metabolite was recovered from the culture filtrate by solvent extraction and was purified by silica gel chromatography using benzene-ethyl acetate (4:1). Pure 2,4-dihydroxy-3,5,6-trimethylbenzoic acid (I), m.p. 192–193°, crystallizes from ethyl acetate-hexane. *Anal.* ($C_{10}H_{12}O_4$): C, 60.68; H, 6.28; O, 31.84; $M^+ = 196$ (mass spectrum); $[\alpha]_D^{25} \pm 0$; λ_{max}^{MeOH} (ε) 264 (11,950) and 310 nm (4410); alkali shifted the maxima to 256 (6940) and 302 nm (5000); ν_{max}^{KBr} 1620, 2860 ($ArCO_2H$) and 3500 cm^{-1} (ArOH); nmr (D_6-DMSO), 7.92 (2x $ArCH_3$), 7.60 ($ArCH_3$), 0.83 τ (broad 3×OH, exch.). The presence of 2 phenolic hydroxyls and a carboxyl was shown by facile formation of a diacetate, m.p. 151–155°; *Anal.* ($C_{14}H_{16}O_6$): C, 59.78; H, 6.13; ν_{max}^{KBr} 1690 ($ArCO_2H$), 1775, 1785 cm^{-1} ($ArOCOCH_3$), nmr ($CDCl_3$) 7.72 and 7.65 τ (2×$ArOCOCH_3$),