in the entire volume of the fibres (figure a). A sample of these fibres was taken for the ion analyses (group 1). The 2nd sample of vacuolated fibres (group 2) was incubated for 30 min again in solution 1 (the procedure is termed "reapplication of glycerol"). 30 min after the start of glycerol reapplication the vacuoles disappeared and the structure of the fibres appeared normal (figure b). The devacuolated fibres (group 2) thus formed the control group. The sodium and potassium concentrations in the fibres were measured with a Perkin Elmer flame photometer and expressed in mmole/kg fibre water. The mean dry weight of fibres was taken as 19%. The cross section of the fibres was considered to be elliptical. Both the major and the minor fibre diameters were measured. All experiments were carried out at room temperature, about 22 °C.

The sodium concentration was significantly higher in vacuolated fibres as compared to devacuolated ones (45.8 mmoles/kg $H_2O \pm 7.3$ SEM and 21.8 mmoles/kg $H_2O \pm 2.0$ SEM, respectively). There was considerable fibre-to-fibre variation of the sodium concentration in vacuolated fibres, which correlated with the structure of the fibres. Intracellular potassium concentration did not show any significant change (see table). The ionic contents in group 2 were close to the values obtained in normal Ringer. The fibres held for comparison during all the experimental time in solution 2 exhibited 25.6 mmoles/kg $H_2O \pm 2.6$ SEM sodium (group 3). This value did not differ significantly from the sodium content of devacuolated fibres. It should be noted that the initial application of glycerol-Ringer did not cause a decrease in the sodium content of the fibres. The mean sodium concentration of the fibres equilibrated in normal Ringer (group 3) and glycerol-Ringer (group 4) was nearly the same (table). The structure of group 3 and 4 fibres was normal, i.e. in both cases no vacuoles were present. The values of sodium concentration obtained on isolated fibres were in good agreement with our data on whole iliofibularis muscles (extracellular space considered equal to inulin space determined on paired muscles). Sodium concentration of whole muscles expressed in mmole/kg fibre water in glycerol-Ringer (2-h-solution 1) was 23 ± 2.8 SEM ($n = 13$), after glycerol removal (2-h-solution 1 → 2-h-solution 2) 41 ± 4.4 SEM ($n = 16$) and after reapplication of glycerol-Ringer (2-h-solution 1 → 2-h-solution 2 → 2-h-solution 1) 24 ± 2.7 SEM ($n = 6$) whereas the control muscles in normal Ringer showed 25 ± 2.0 SEM ($n = 18$) sodium. It is possible that high Na concentrations in group 1 fibres may be due to the presence of numerous vacuoles in these fibres. It has been shown that the vacuoles formed from TTS by glycerol removal contain the extracellular marker ferritin (11 nm diameter) when ferritin is present in the bathing solution, both before and during the washing out of the glycerol[2]. After the vacuoles appeared they were found to be inaccessible to ferritin[2]. As free communication exists between the extracellular fluid and the lumen of TTS, it may be suggested that the increased NaCl in the vacuoles comes from the extracellular fluid. In order to account for the approximately 2-fold rise in sodium concentration in group 1 fibres the volume of the vacuoles produced by glycerol withdrawal must be about 20% of the total volume of the fibre (assuming that the concentration of NaCl inside the vacuoles is the same as that in extracellular fluid). This volume is rather close to that (15%) determined in isolated muscle fibres of the crayfish[5].

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Biological activity of the C-terminal octapeptide of cholecystokinin, of three of its analogues and of caerulein in the dog[1]

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Summary. Dose-response curves of the C-terminal octapeptide (CCK-8) of cholecystokinin, of 3 of its methoxinine analogues, and of caerulein for various variables of exocrine pancreatic secretion have been established in conscious dogs. The following relative potencies were calculated for the protein secretion activity of CCK-8 (100%), [Mox^3]-CCK-8 (52%), [Mox^6]-CCK-8 (27%), [Mox^3,Mox^6]-CCK-8 (19%) and caerulein (178%).

Cholecystokinin (CCK) is a tritriacontapeptide bearing a sulfate ester group on its tyrosine residue. Due to its complicated structure its synthesis has not yet been accomplished. Since it has been found that the biological activities of small fragments of the C-terminal end of CCK, like the octapeptide (CCK-8)[2,3], and structurally related peptides like caerulein[4] are qualitatively similar to those of the larger molecule, they are used for biological studies as well as for diagnostic purposes. The interest in these peptides has further been enhanced by the recent observation that

ED_{50} values and relative potencies of CCK-8, its analogues and caerulein for exocrine pancreatic secretion

Compound		ED_{50} pmoles/kg · h (relative potency)					
		Volume		Protein output		Bicarbonate output	
A	Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (CCK-8)	77	(1.00)[a]	40	(1.00)[a]	79	(1.00)[a]
B	Asp-Tyr(SO_3H)-Mox-Gly-Trp-Met-Asp-Phe-NH_2 ([Mox^3]-CCK-8)	92^{NS}	(0.83)	79*	(0.52)	116^{NS}	(0.67)
C	Asp-Tyr(SO_3H)-Met-Gly-Trp-Mox-Asp-Phe-NH_2 ([Mox^6]-CCK-8)	128*	(0.60)	152**	(0.27)	166*	(0.47)
D	Asp-Tyr(SO_3H)-Mox-Gly-Trp-Mox-Asp-Phe-NH_2 ([Mox^3, Mox^6]-CCK-8)	194**	(0.39)	221**	(0.19)	243**	(0.32)
E	Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 (caerulein)	37	(2.10)	22	(1.78)	50	(1.57)

[a] Relative potency with respect to A. * $p < 0.05$; ** $p < 0.01$; NS = no significance (significance of the differences with respect to A).

they occur as such in the gut and brain of several species and may themselves play a physiological role[5,6].

Structure-activity studies by others[7,8] have shown that the methionine residue in the C-terminal region of CCK-8 and caerulein is crucial for biological activity, since its replacement by any residue other than the closely related norleucine residue results in a great loss of potency. To learn what effect replacement of methionine by its oxygen analogue methoxinine (Mox, i.e. α-amino-γ-methoxybutyric acid)[9] would have on biological activity we synthesized [Mox^3]-CCK-8, [Mox^6]-CCK-8 and [Mox^3,Mox^6]-CCK-8[10]. We here report their biological activity with respect to exocrine pancreatic secretion in comparison with CCK-8 and caerulein in conscious dogs.

Material and methods. Caerulein was kindly provided by Farmitalia, Freiburg, FRG. The synthesis and in vitro activities of CCK-8 and its analogues have been described elsewhere[10]. 4 dogs were operatively equipped with gastric and duodenal fistulae by means of modified Thomas cannulas, which permit drainage of gastric juice and direct cannulation of the main pancreatic duct. The animals were allowed to recover from surgery for at least 6 weeks. The dogs were fasted 18 h prior to the experiment. The 5 substances were i.v. administered to the 4 dogs in a randomized order. To each of the 4 dogs each of the 4 doses, 60, 90, 135, and 210 pmoles/kg · h, were successively infused over 30 min. Corresponding to its higher potency[11], caerulein was given in doses providing pancreatic responses similar to those obtained with CCK-8, i.e. 20, 30, 45, and 70 pmoles/kg · h. Pure pancreatic juice was collected in 10-min samples. Volume was measured to the nearest 0.25 ml, total protein concentration was determined by the biuret reaction and bicarbonate concentration by backtitration with NaOH after the addition of HCl and heating the sample[12].

Statistical analysis. In order to estimate the relative potencies of the various compounds, 4 parameter logistic functions were fitted to mean values by the method of least squares. The logistic function is described by the following formula:

$$f(c) = \gamma + \frac{\delta - \gamma}{1 + e^{-\beta(\ln c - \ln \mu)}}$$

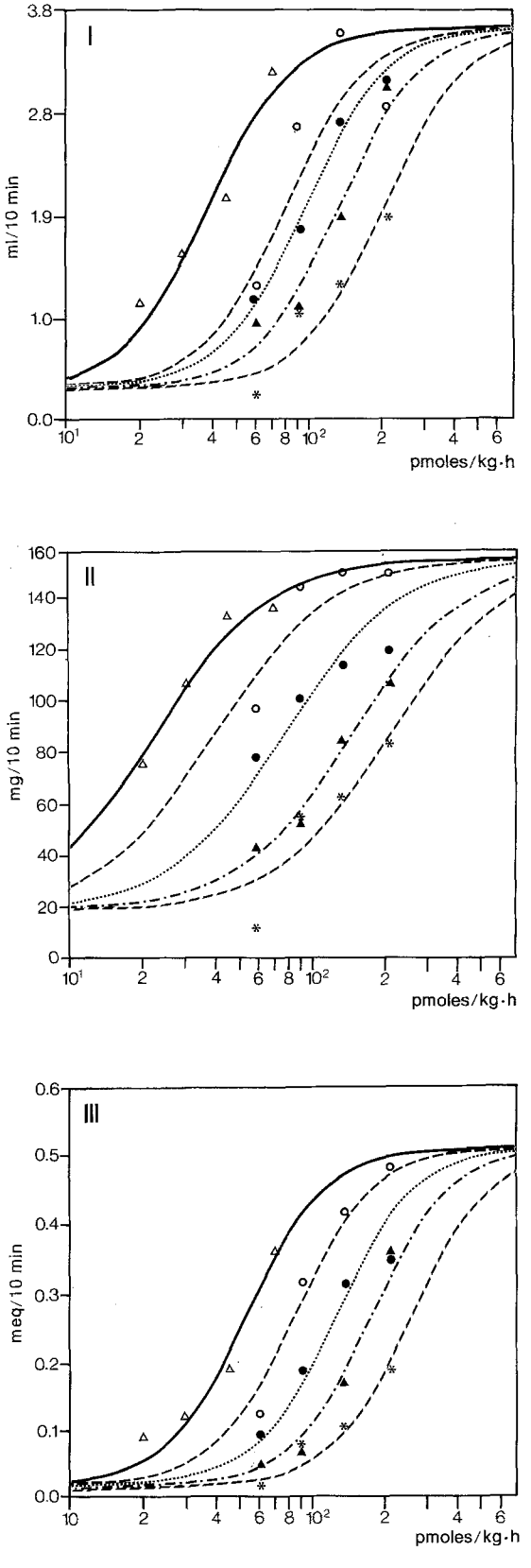
in which

$f(c)$ = effect of preparation at dose c,
γ = value of the 'basic response' (control value),
δ = value of the maximal response,
β = a measure of the slope of the curve,
μ = dose producing the half maximal effect.

The relative potency of a preparation B with respect to a preparation A is given by the ratio of the μ-values ($\mu A/\mu B$). For this calculation it was assumed that the dose-response curves of the 5 preparations were parallel and that the basic and maximal responses were identical, i.e. β, γ, δ were assumed to be the same for all preparations. Note that the Michaelis-Menten equation, which has been used for the analysis of similar experiments[13] can be considered to be a special 4 parameter logistic function with $\beta = 1$. The tests of significance were performed by comparing in each dog the mean values of the results gained with the 4 different doses using the least significant difference procedure[14].

Results. The results are shown in the figure. Each dot represents the arithmetic mean of the data obtained from 4 dogs. In addition the fitted logistic functions are plotted. According to these plots the mathematical model seems to be consistent with experimental results.

The calculated ED_{50} and the relative potencies of the peptides as compared to CCK-8 are given in the table. For all 3 parameters the sequence of potencies of the 5 sub-



Dose-response curves of CCK-8, its analogues and caerulein for pancreatic volume (I), protein output (II), and bicarbonate output (III). Each dot represents the mean of the results obtained in 4 dogs. The curves represent the fitted logistic functions. △——△ Caerulein; ▲- · - · - ·▲ [Mox^6]-CCK-8; ○— — —○ CCK-8; ✱— — —✱ [Mox^3,Mox^6]-CCK-8; ●·····●[Mox^3]-CCK-8.

stances is the same. E (caerulein) is the most potent stimulant followed by A (CCK-8), B, C and D. In an additional experiment the analogues were administered in high doses up to 3600 pmoles/kg · h. Under these conditions their effect was similar to the maximal response obtained with CCK-8.

Discussion. CCK-8, like CCK, stimulates mainly pancreatic protein secretion; volume and bicarbonate are stimulated to a much lesser degree. None of the 3 analogues reached the potency of CCK-8 for any parameter of exocrine pancreatic secretion. The substitution of methionine by methoxinine in position 3 (analogue B) already weakens the potency of the octapeptide. This is in contrast to the in vitro results obtained on guinea-pig gall bladder strips in which this analogue showed almost the same EC_{50} as CCK-8[10]. The substitution in position 6 (analogue C) induces a considerable reduction of the biological activity, and consequently with double substitution in positions 3 and 6 most of the activity is lost (analogue D). This reduction was even more pronounced in the in vitro assays[10], where analogues C and D showed only 5 and 2% respectively of the CCK-8 potency. However the sequence of the analogues with respect to potency is the same in both systems. Since all compounds produced the same maximal effect they must be considered full agonists with respect to exocrine pancreatic secretion, differing from each other mainly in their affinity for the receptor. This conclusion is fully supported by the in vitro studies.

The decapeptide caerulein was, on a molar basis, 1.8 times more potent than CCK-8 in stimulating pancreatic protein secretion. This is somewhat lower than the factor 3 published previously by Grossman's group[11].

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Supersensitivity after intraventricular 6-hydroxydopamine: Relation to dopamine depletion[1]

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Summary. 6-Hydroxydopamine increased behavioral response to L-DOPA in proportion to the decrease of dopamine (DA) and DA uptake in rat striatum. The increased response to apomorphine, however, only occurred after > 80% DA loss. Thus, 6-hydroxydopamine may induce postsynaptic changes only following large lesions.

Animals sustaining large lesions of central dopamine (DA) - containing neurons show increased responsiveness in various behavioral tests to certain dopaminergic agonists, including L-DOPA and apomorphine[2–8]. In rats with different magnitudes of DA depletion, produced by intraventricular administration of the neurotoxin, 6-hydroxydopamine (6-HDA), we have recently found that an increased response to L-DOPA occurred even when the loss of DA was relatively small, whereas an increased response to apomorphine required a large depletion[7,8]. We now explore the basis for these observations and discuss their implications for the recovery of function seen in rats after damage to central dopaminergic neurons.

Male Sprague-Dawley rats (Zivic-Miller Labs) weighing 200–250 g at the start of each experiment were house singly and given food pellets and water ad libitum. 6-HDA hydrobromide (50–250 µg, doses expressed as units of free base) or its vehicle (0.9% NaCl, 0.1% ascorbic acid) was administered into the lateral ventricles[9]. 30 min prior to 6-HDA or vehicle injections, animals were given pargyline (50 mg/kg, i.p.), to potentiate the effects of 6-HDA on dopaminergic terminals, and desmethylimipramine (25 mg/kg, i.p.), to protect noradrenergic terminals from destruction. Most rats appeared normal within 1–2 days. However, some animals receiving the higher doses (200–250 µg) became aphagic and required intragastric intubation of liquid diets and/or access to highly palatable foods for several days before they could maintain their body weight on pelleted chow and water. Behavioral and biochemical measurements were made 40–60 days after lesioning.

When treated with L-DOPA (60 mg/kg, i.p.), most of the 6-HDA-treated animals were more responsive than control rats. In contrast, only animals receiving the largest amounts of 6-HDA were at all responsive to apomorphine (0.05 mg/kg, i.p.). When DA depletions (measured 1 week later[10]) were compared to performance during this behavioral test, the motor activity induced by L-DOPA was found to increase with increasing loss of DA throughout a broad range of brain damage, while an increased response to apomorphine was seen only in animals whose DA depletions were greater than 80% (figure 1). These results confirm and extend our previous studies in which lesion-induced changes in the dose-response curves for these agonists were examined[7,8].

In a 2nd group of rats we assessed high affinity DA uptake[11], an index of DA transport, or DA-stimulated adenylate cyclase[12], an index of target cell responsiveness.