of thyroid activity[11]. The 20% increase in the height of the follicles, in the present study, indicates hyper thyroid activity. Hyperthyroidism results in a syndrome known as 'exopthalmos', the symptoms being bulging eyeballs with signs of hyperexcitability[12]. However, exopthalmos has also been reported in the trout infected with a virus known as VHS[13]. Apart from exopthalmos, no symptom associated with viral infection could be detected in the present study. Therefore, the exopthalmos observed here may be attributed to hyperthyroidism. Copper-induced hyperthyroidism, resulting in exopthalmos, has not hitherto been reported in any fish.

The appearance of exopthalmos in only 2 out of 10 copper concentrations could only be explained on the presumption that, perhaps, the lower concentrations are too weak to act on the nervous system to activate the thyroid through the hypophysis, and that the higher concentrations are strong enough to cause quick and intensive brain damage, thereby preventing activation of the thyroid. It is well known that the brain exerts control over many functions of the hypophysis, including production and release of TSH, which in turn activates production of thyroid hormones[11]. Consequently, any damage to the nervous system may possibly stop thyroid stimulation. The neurotoxic effects of copper on fishes are already well documented[14,15].

Thus it appears that 0.750 and 1.000 mg/l of copper are optimum doses for the fry of *Ophiocephalus punctatus*, for triggering the hypothalamo-hypophysial-thyroid mechanism, resulting in hyperthyroidism. However, further studies employing sophisticated techniques, for which facilities do not exist with us, are needed in order to understand fully the process of thyroid activation by copper.

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Effect of enkephalins in the presence of the antibiotic bacitracin in the longitudinal muscle strip preparation from guinea-pig ileum

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Summary. The antibiotic bacitracin (5×10^{-5}–4×10^{-4} M) increases the inhibition of the contractile response caused by both enkephalin release and direct application of Met-enkephalin 5×10^{-7} M in the longitudinal muscle strip preparation from guinea-pig ileum. This effect is attributed to an inhibition of enkephalin degrading peptidases by bacitracin.

Bacitracin is a peptidase-inhibiting antibiotic which prevents enzymatic peptide degradation. This characteristic of bacitracin was investigated by Schulz et al. in 1977[1], using longitudinal muscle strips from guinea-pig ileum. When electrically stimulated, this preparation releases enkephalins[1-3]. These polypeptidic substances are very quickly enzymatically degraded in vivo as well as in vitro[4]. The addition of bacitracin should increase the enkephalin concentration in the medium[1]. However, the results were not the expected ones; addition of 2×10^{-5} M bacitracin caused a marked decrease of enkephalinergic material released by electrical stimulation. (This material was detected by TLC.) Consequently the authors mentioned suggested a possible interaction of bacitracin with the enkephalin release mechanism or, as a more likely explanation, with the mechanism by which opiate-like material is formed from a larger peptide through enzymic cleavage. This precursor would lack opiate-like activity and was not detected by the methods used.

In the present investigation, we have provoked a massive enkephalinergic release from longitudinal muscle strips from guinea-pig ileum by means of 10-Hz tetanizing shocks, according to the method described by Puig et al. in 1977[2]. Met-enkephalin and morphine have also been administered directly, and the effect of bacitracin on the phenomena observed has been investigated.

Materials and methods. The myenteric plexus-longitudinal muscle strip from guinea-pig ileum was prepared as described by Paton and Zar[5]. Each strip was suspended in a 40-ml organ bath containing Krebs-Henseleit[6] solution at 32 °C bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The isometric contractions of the muscle were recorded by means of a force transducer coupled to a Ugo Basile polygraph. The tension of the strip was maintained at 1 g. The tissue was stimulated by field stimulation through 2 platinum ring electrodes with a basic 0.3 Hz, 2-msec electric stimulus and supramaximal voltage ($\simeq$ 60 V). A Grass SD9 stimulator was used. 5 min after the bacitracin (2.5×10^{-5} M–4×10^{-4} M) administration, a tetanizing shock was delivered by increasing the stimulus frequency up to 10 Hz for 5 min and then returning to the basic stimulus conditions with 0.3 Hz.

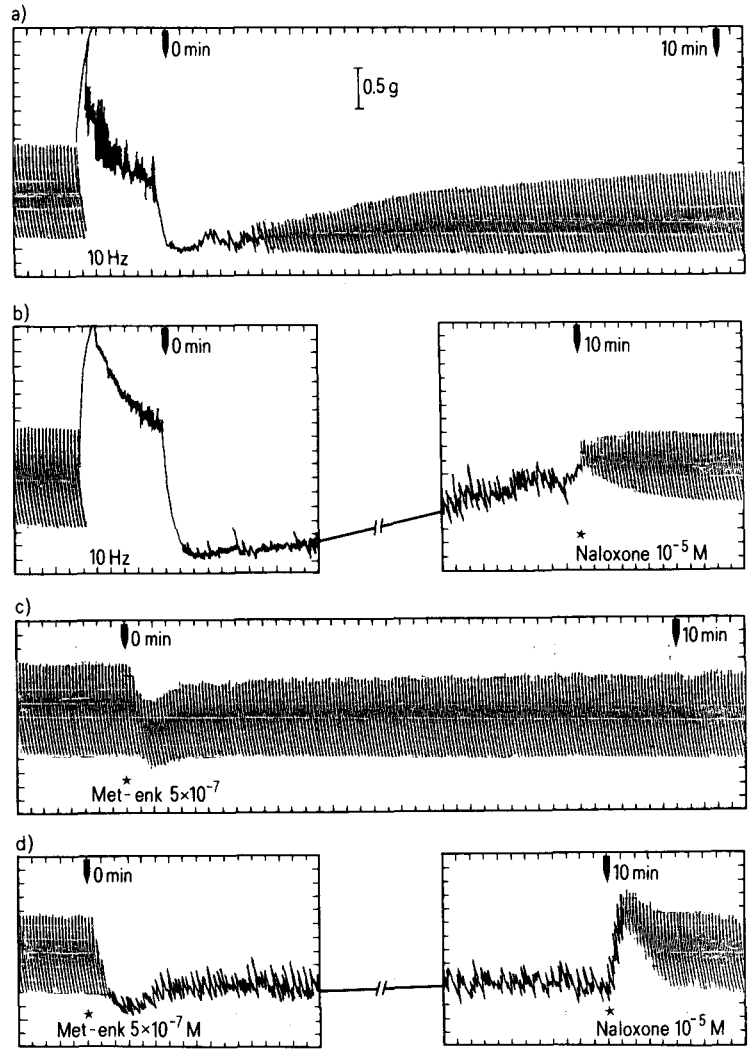
Influence of bacitracin on contraction recovery after a 10-Hz tetanizing shock or after application of 5.10^{-7} M Met-enkephalin in the longitudinal muscle strip preparation from the guinea-pig ileum

Bacitracin	10-Hz shock Recovery (%)		Met-enkephalin Inhibition (%)		Recovery (%)	
0 (Control)	91.7± 10.1	(6)	35.7±5.5	(11)	88.8±4.1	(11)
2.5×10^{-5} M	75.8±4.1	(6)	56.2±6.2*	(6)	78.5±4.5	(6)
5×10^{-5} M	63.6±3.0*	(5)	72.3±5.9***	(6)	69.5±6.0*	(6)
10^{-4} M	59.6±6.3*	(5)	70.8±9.1**	(6)	64.3±9.5*	(6)
2×10^{-4} M	33.8±4.9**	(5)	75.0±6.7***	(6)	58.5±5.7***	(6)
4×10^{-4} M	15.6±9.8***	(5)	74.0±9.8**	(6)	43.7± 14.1**	(6)

% Recovery was estimated by comparing the amplitude of the contractile response 10 min after the end of the 10-Hz pulse with the one before the pulse. The results are expressed as mean±SE. Number of test performed in parantheses. * Statistically significant difference with respect to the control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Student's two-tailed t-test for unpaired data).

In a 2nd batch of longitudinal muscle strips, 5×10^{-7} M Met-enkephalin was administered either in the presence or in the absence of bacitracin (2.5×10^{-5} M–4×10^{-4} M). Likewise, a possible bacitracin interaction with the morphine effect was investigated. We performed parallel trials adding a 2×10^{-7} M concentration of morphine in the presence and in the absence of 4×10^{-4} M bacitracin.

Results. The administration of bacitracin (2.5×10^{-5}–4×10^{-4} M) did not modify the response of longitudinal muscle strips to the basic stimulation with 0.3 Hz. However, stimulation of the preparation with 10 Hz caused an inhibition of the contractile response to subsequent stimulation with 0.3 Hz. Recovery of the response amplitude was quick; 10 min after the end of the tetanizing stimulation, the contraction height was 91.7± 10.1% of the initial response (fig. A). Recovery of the response in the presence of bacitracin was incomplete. A relationship between the concentration of bacitracin and the percent of recovery of the contractile response was found (table). After administration of the highest bacitracin dose used (4×10^{-4} M),



Response of the longitudinal muscle strip preparation to a 0.3-Hz, 2-msec and supramaximal voltage stimulation: A) Control response after a 10-Hz stimulation. B) Response after a 10-Hz stimulation in the presence of bacitracin 4×10^{-4} M followed by naloxone reversion 10 min later. C) Effect of a Met-enkephalin 5×10^{-7} M administration. D) Effect of a Met-enkephalin 5×10^{-7} M administration in the presence of bacitracin 4×10^{-4} M. Naloxone reversion 10 min later. – A recording chart when basic 0.3-Hz stimulation corresponds to 15 sec. When a 10-Hz stimulation took place the recording rate was reduced (1 square = 1 min).

naloxone could still reverse the inhibition caused by the enkephalin release within 10 min (fig. B).
When 5×10^{-7} M Met-enkephalin was administered during electrical stimulation with 0.3 Hz, an inhibition of $35.7 \pm 5.5\%$ of the contractile response took place. However, after 10 min the contraction amplitude was again $88.8 \pm 4.1\%$ of the initial contractile response (fig. C).
Bacitracin (4×10^{-4} M) increased the inhibition produced by Met-enkephalin and prolonged the effect of this inhibition (table; fig. D). In this case too, naloxone was able to reverse the inhibition caused by Met-enkephalin application (fig. D).
The inhibitory effect of morphine was not affected significantly by the presence of bacitracin (4×10^{-4} M) in the organ bath. Control inhibition was $37.2 \pm 4.1\%$, and inhibition in the presence of bacitracin was $29 \pm 2.8\%$ ($n = 5$).
Discussion. Even when bacitracin seemed to increase the recovery of enkephalins the values detected by some authors[7,8] were not statistically significant. The necessity of finding means to protect these peptides better from degradation has been emphasized; the bacitracin concentration that they used might not fully have protected enkephalins from degradation. For this reason, we tentatively used higher bacitracin concentrations. By doing so, we have been able to show that bacitracin protects both endogenous enkephalins released from longitudinal muscle strips from guinea-pig ileum by electric stimulation and exogenous enkephalins directly added into the organ bath. Moreover, a relationship between the duration of the enkephalin effect and the bacitracin concentration in the organ bath could be established (table).
We conclude from the present results that bacitracin is able to inhibit enkephalin-degrading peptidases. In the longitudinal muscle strip preparation this inhibition is already significant at bacitracin concentrations 2.5 times higher than those used by Schulz et al.[1].
Finally, bacitracin does not affect the inhibition caused by morphine and this demonstrates its specific protective action against the degradation of opiate polypeptidic drugs.

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Effect of abrupt withdrawal of chronically administered β-blocking drugs on cardiac sensitivity in the rat

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Summary. Increased tachycardia to isoprenaline was observed in pithed rats 2 days after withdrawal of propanolol but not after withdrawal of atenolol (a cardioselective drug) or LL 21-945 (a long acting beta-blocking drug).

Abrupt cessation of therapy with β-adrenoceptor blocking drugs often provokes severe angina, ventricular arrhythmias and myocardial infarction. This phenomenon is now well accepted and has been termed the β-blockade withdrawal syndrome.
The simplest explanation of the condition is that physical activity that was tolerated during therapeutic protection with β-blockade was continued after cessation of treatment and thus symptoms were precipitated. However, the occurrence of withdrawal effects in resting, hospitalized patients[2] would indicate another cause. Further attempts to explain the phenomenon suggest rebound effects of the actions of β-blockers in decreasing platelet reactivity to aggregating agents[3], decreasing plasma renin activity[4] and alteration of haemoglobin-oxygen affinity[5]. The similarity of some of the withdrawal symptoms to those of hyperthyroidism has also prompted the suggestion of the implication of triiodothyronine[6].
The well established experimental observation of denervation supersensitivity provides the most acceptable explanation of the β-blockade withdrawal syndrome. Increased sensitivity of organs occurs not only after surgical denervation, but also after drug-induced reduction in nerve activity ('chemical denervation') with ganglion or adrenergic neurone blocking drugs. Further, receptor blocking drugs can induce supersensitivity. This is illustrated by the increase of salivation following cessation of prolonged atropine treatment[7].
Preliminary evidence for such an increase in myocardial sensitivity to sympathomimetics after β-blockade withdrawal has been obtained from in vitro[8] and in vivo experiments[9] and in clinical studies in human hypertensive patients[10]. However, these observations were not confirmed in healthy volunteers[11], dogs[12] and rabbits[13].
The present work extends earlier observations with propranolol in rats[9] and examines 2 further β-blocking agents, a very long-acting drug, LL 21-945[14] and a cardioselective drug, atenolol.
Materials and methods. Male rats (Chelsea-Wistar strain) approximately 190–200 g initial b.wt were anesthetized with ether and a tracheal cannula inserted. The brain and spinal cord were destroyed by passage of a narrow metal rod via the right orbit into the cranium and thence down the spinal cord. Artificial respiration was maintained by a positive pressure pump. Blood pressure was recorded from a carotid cannula via a transducer to a pen recorder. Heart rate was recorded by a rate meter triggered by the systolic pulse.
Tachycardias to gradually increasing femoral i.v. doses of isoprenaline hydrochloride were measured and the maximum response was obtained. The dose of isoprenaline causing 50% of the maximum (ED 50) was measured from the log dose-effect curve. Significance of changes were established by the Mann-Whitney U-test.
Measurements were made on control animals and on rats that had been given propranolol (50–60), LL 21-945 (2–3) or atenolol (60–70 $mg \cdot kg^{-1}\ day^{-1}$) in their drinking water