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.

1 R. Schulz, M. Wuster, R. Simantov, S. Snyder and A. Herz, Eur. J. Pharmac. *41*, 347 (1977).
2 M.M. Puig, P. Gascon, G.L. Graviso and J.M. Musacchio, Science *195*, 419 (1977).
3 J. Hughes, in: Receptors vol. 1, p. 31. Proc. 7th int. Cong. Pharmac. Paris. Pergamon Press, London 1978.
4 J.L. Meek, H.Y.T. Yang and E. Costa, Neuropharmacology *16*, 151 (1977).
5 W.D.M. Paton and A.M. Zar, J. Physiol. *194*, 13 (1968).
6 H.A. Krebs and Z.H. Henseleit, J. phys. Chem. *210*, 33 (1932).
7 L.L. Iversen, D.S. Iversen, F.E. Bloom, T. Vargo and R. Guillemin, Nature *271*, 679 (1978).
8 J.A. Richter, D.L. Wesche and R.C.A. Frederickson, Eur. J. Pharmac. *56*, 105 (1979).

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

for 11–13 days. Animals were caged in pairs and water (or drug solution) intake was measured daily. In some animals adrenal gland and heart weights were recorded.

Results. Withdrawal of propranolol resulted in an insignificant increase in sensitivity to isoprenaline 1 day after withdrawal. A 4-fold increase ($p < 0.002$) was observed 2 days after withdrawal. With neither LL 21-945 (up to 9 days off) or atenolol (up to 5 days off) was any increased sensitivity observed (fig.). Metoprolol in doses up to 100 $mg \cdot kg^{-1}$ day^{-1} failed to produce a significant blockade of isoprenaline. It was assumed therefore to be poorly absorbed in this strain of rats.

Whereas no control rats died during the experimental procedure 25% of the 2-day propranolol withdrawn rats died shortly after pithing. In those animals that died pink fluid was often exuded from the lungs.

2 rats treated with propranolol then withdrawn for 2 days manifested an inordinate supersensitivity (300 and 750× control values). However, since these lost weight (10 g) rather than gaining approximately 30 g during the experimental period these results were not included. There was no difference in heart or adrenal gland weight or in resting heart rates between control or test animals.

Discussion. The measurement of the response of the heart to adrenoceptor agonists in the pithed rat is a satisfactory way to monitor possible changes in cardiac sensitivity. It has the advantage of being an in vivo preparation but is uncomplicated by reflex nervous influences. It would be more rational to measure sensitivity to the actual transmitter, noradrenaline, rather than isoprenaline. This proved difficult since, particularly in the treated and withdrawn animals, the pressor effects of noradrenaline precipitated heart failure after injection of the high doses used to establish to maximum responses. With the depressor action of isoprenaline this problem was not encountered.

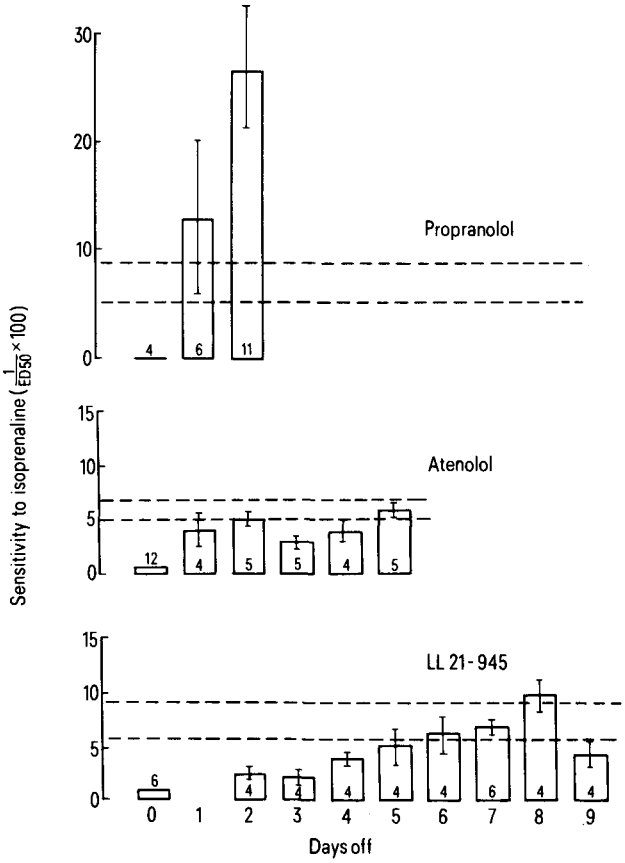
The 3 β-blocking drugs used produced a substantial blockade of the isoprenaline effects. After withdrawal of propranolol an increase in sensitivity to isoprenaline was apparent within 24 h although this was not statistically significant due to the high scatter of the results. By 2 days, however, a 4-fold increase in sensitivity was observed. It is postulated that this is a reflection of an intrinsic increased sensitivity of the myocardium to the β-adrenoceptor agonist. Although corticosteroids can increase sensitivity to sympathomimetic drugs[16] there was no change in adrenal weight in the treated animals. The participation of thyroid hormones in this rebound effect[6] is unlikely since there was no increase in the resting heart rate of the treated animals.

The most likely explanation of the rebound supersensitivity observed is that the prolonged β-adrenoceptor blockade results in an increase in number of receptors. Glaubiger and Lefkowitz[15] noted a doubling of the number of dihydroalprenolol binding sites after 14 days i.p. dosing with propranolol (10 $mg \cdot kg^{-1}$) a regime not very different from that described in this paper given the greater effect of parenteral rather than oral administration. Such a rebound myocardial supersensitivity has not always been shown experimentally[11,13] and the reason for this may be the inherent variability implicit in such experiments where differences in degree of absorption, rate of elimination and duration of treatment and withdrawal all combine to increase the scatter of results.

Rebound supersensitivity was not observed with LL 21-945. The duration of action of this drug is long, a single dose in dog still retains half of its β-blocking activity 39 h after i.v. injection[14]. The absence of withdrawal effects is presumably due to the fact that the slow reversal of blockade is paralleled by a decay of supersensitivity.

The lack of rebound sensitivity following withdrawal of the cardioselective blocking drug atenolol is less easy to explain. It is unlikely to be due to slow decay of blockade since its half-life (6–9 h) is not so greatly different to that of propranolol (4–6 h). It was not, however, possible to achieve as great a block of isoprenaline with the atenolol dose regime as with propranolol and it may be that the intensity of the withdrawal effect is a reflection of the degree of β-blockade. It is also possible that the sensitivity is not so easily produced by a cardioselective blocking drug and that β_2-receptor blocking action is necessary to induce this effect. It is perhaps relevant that the β-adrenergic-induced cardiac hypertrophy is inhibited by propranolol but not by selective β_1-receptor blocking drugs[17].

The pathological effects following cessation of chronic administration of propranolol, if it is a general phenomenon explicable in terms of increase in β-receptor number or sensitivity, has profound implications. It is obvious that abrupt cessation of treatment either by design or accidental non-compliance by patients is to be avoided. In addition, if the therapeutic effect of β-adrenoceptor blocking agents is mediated by β-blockade, as suggested by Himori et al.[18], the treatment, for instance, of mild hypertension by β-blockers could actually aggravate hypertension after withdrawal of treatment due to development of β-adrenoceptor supersensitivity.



Sensitivity of pithed rats to chronotropic effects of i.v. injection of isoprenaline during, and at various times after withdrawal of treatment with propranolol (50–60), atenolol (60–70) and LL 21-945 (2–3 $mg \cdot kg^{-1}$ day^{-1}). Dotted lines represent the limits of the standard error round the mean response of control rats (14 for each experiment). Increased sensitivity was detected 2 days after withdrawal of propranolol ($p < 0.002$) but not following withdrawal of the cardioselective drug atenolol or the long acting β-adrenoceptor blocking drug LL 21-945. Figures in columns refer to numbers of rats used.

1 Acknowledgments. We are grateful to Sandoz Ltd for a gift of LL 21-943 and to I.C.I. for gifts of propranolol and atenolol.
2 T. Meinertz, H. Just, W. Kaspar, F. Kersting and K.-H. Brening, Lancet *1*, 270 (1979).
3 W.H. Frishman, B. Weksler, J.P. Christodoulou, C. Smithen and T. Killip, Circulation *50*, 887 (1974).
4 A.M. Michelakis and R.G. McAllister, J. clin. Endocr. *34*, 386 (1972).
5 F.A. Oski, L.D. Miller, M. Delwaria-Papactopoulos, J.H. Manchester and J.C. Shelburne, Science *175*, 1372 (1972).
6 B. Kristensen, E. Steiness and J. Weeke, J. clin. Pharmac. Ther. *23*, 624 (1978).
7 N. Emmelin, Experientia *21*, 57 (1965).
8 G. Kunos, B. Robertson, W.H. Kan, H. Preiksaitis and L. Mucci, Life Sci. *22*, 847 (1978).
9 J.H. Botting and A. Gibson, Lancet *1*, 875 (1979).
10 H. Boudoulas, R.P. Lewis, R.E. Kates and G. Dalamangas, Ann. intern. Med. *87*, 433 (1977).
11 J.A. Pantano and Y.C. Lee, Archs intern. Med. *136*, 867 (1976).
12 J.H. Myers and L.D. Horwitz, Circulation *58*, 196 (1978).
13 A.E.G. Rain and E.M. Vaughan Williams, Br. J. Pharmac. *70*, 205 (1980).
14 B.J. Clark, K. Saameli and F. Troxler, Br. J. Pharmac. *52*, 123P (1974).
15 G. Glaubiger and R.J. Lefkowitz, Biochem. biophys. Res. Commun. *78*, 720 (1977).
16 O. Pathomvanich, Ph.D. Thesis, University of London 1970.
17 J.R. Womble, D.R. Larson, J.G. Copeland, B.R. Brown and D.H. Russell, Proc. 8th int. Cong. Pharmac., 1981.
18 N. Himori, T. Ishimori and N. Taira, Archs int. Pharmacodyn. Ther. *242*, 115 (1979).

Manganese prolongation of pentobarbital hypnosis in the male rat[1]

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Summary. Following manganese treatment, pentobarbital hypnosis was prolonged in male rats. The maximal effect occurred from 1 to 3 days following manganese treatment and the threshold dose was found to be 3 mg Mn^{++}/kg (i.p.).

Manganese is a physiologically essential trace metal in both man and animals, forming an integral part of the metalloenzymes, pyruvate carboxylase[2] and mitochondrial superoxide dismutase[3]. However, chronic or acute exposure to high levels of this metal can lead to various toxic syndromes[4] including hepatic damage[5,6].

The administration of other trace metals, such as lead[7] and cadmium[8], produces hepatic effects such as inhibition of drug biotransformation which leads to an altered responsiveness to several drugs including the barbiturates. This study was undertaken to examine the effect of acute manganese administration on drug response in the male rat.

Methods. Male, Sprague-Dawley rats, weighing 140–160 g, were obtained from Sasco, Inc. (Omaha, NE) and housed in community cages for at least 1 week prior to use. Animals were maintained in environmentally controlled rooms at approximately 22 °C under a 12/12 h (L:06.00–18.00 h) alternating light-dark cycle with free access to food (Purina Rat Chow, Ralston Purina Company, St. Louis, MO) and tap water.

Manganese ($MnCl_2 \cdot 4\ H_2O$) and pentobarbital Na solutions for injection were prepared using distilled, deionized water such that each animal received 1 ml/kg b. wt, i.p. The duration of hypnosis was defined as the time from loss to recovery of the righting reflex.

Statistical analyses were performed using an analysis of variance (ANOVA) followed by Duncan's New Multiple Range test[9] where appropriate. The acceptable level of significance was established at $p=0.05$.

Results. Preliminary studies in this laboratory showed that a manganese dose of 10 mg Mn^{++}/kg (i.p.) significantly prolonged the duration of pentobarbital hypnosis. Therefore, this dose was used to examine the time-course of manganese effect on drug response. Animals were treated with manganese at various time periods, ranging from 1 to 10 days, prior to

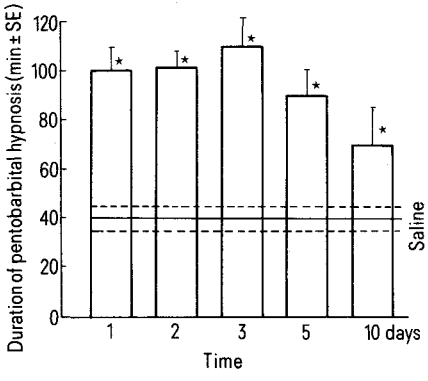

Figure 1. Time-course of manganese prolongation of pentobarbital hypnosis in male rats treated with manganese (10 mg Mn^{++}/kg, i.p.). At the specified time intervals animals received pentobarbital Na (35 mg/kg, i.p.) and the duration of hypnosis was determined. Controls received normal saline ten days prior to pentobarbital. *Significantly different from control animals ($p < 0.05$).

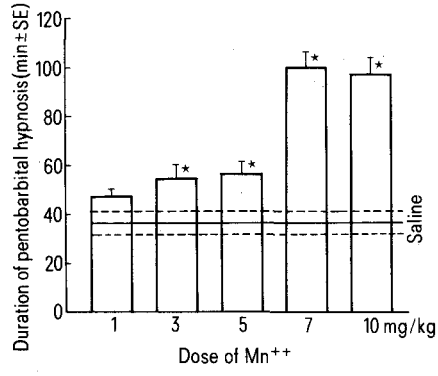

Figure 2. Dose-response of manganese prolongation of pentobarbital hypnosis in male rats. Male rats were treated with manganese (1–10 mg Mn^{++}/kg, i.p.) and 3 days later animals received pentobarbital Na (35 mg/kg, i.p.) and the duration of hypnosis was determined. *Significantly different from control animals ($p < 0.05$).