in vitro conditions now used, the atria responded markedly to PHE and this response was significantly lowered after a week's cold exposure. It can thus be concluded, supported by the findings from frogs and toads[8,11], that this lowered sensitivity results from a decreased sensitivity of cardiac α-adrenoreceptors. Furthermore, it is reasonable to assume that the enhanced sympathetic activity of cold-exposed rats is responsible for this subsensitivity of α-receptors. This assumption is supported by the findings that the decreased sympathetic activity in rats resulting from decentralization or from 6-hydroxydopamine treatment causes supersensitivity to NA but not to ISO[12,13]. On the other hand, sympathetic hyperinnervation caused by the treatment with nerve growth factor results in subsensitivity to NA when measured in an isolated mouse intestine[14]. Actually, the increase in NA release at the beginning of cold acclimation[15] is related temporally to the subsensitization of the α-adrenoreceptors found in this study. However, it still remains obscure why the sensitivity subsequently returns although the higher NA release still continues.

The present results further show that, after prolonged cold acclimation, the maximum response increased to ISO and to NA but not to PHE. It has been found that cold-acclimated rats show a striking increase in their metabolic response to NA and to ISO, due to an increased capacity of the β-receptors to respond rather than to increased sensitivity[16,17]. It is the higher level of NA in cold-exposed rats which results in this increased β-response[1,2]. Thus the increased maximum response to ISO and to NA found in the present study after prolonged cold acclimation can be regarded as an increased β-response, due to an increased release of NA from the sympathetic nerve endings.

The prolonged cold acclimation also caused another significant change in response to NA. The sensitivity of atria to NA was markedly increased after 40–45 days of cold acclimation. This type of supersensitivity was not found to ISO or to PHE, which probably means that neither β- nor α-receptors are involved. This supersensitivity to NA could be explained by a reduction in NA uptake, because the affinity of NA for the uptake process is much greater than that of either ISO or PHE[18]. This explanation, however, is unsatisfactory on the basis that a higher level of NA (or ISO) in the organism, as produced by repeated injections, did not affect the activity of the uptake process in the rat heart[19]. Thus, the mechanism(s) involved in this sensitization still have to be elucidated in further experiments.

Zusammenfassung. Eine Woche Kälteadaptation vermindert die Frequenz der isolierten Rattenherz-Vorkammern und reduziert ihre chronotrope Empfindlichkeit besonders gegen Phenylephrin, weniger gegen Noradrenalin, und gar nicht gegen Isoprenalin. Hingegen verstärkte eine 6wöchige Kälteadaptation die maximalen Effekte von Isoprenalin und Noradrenalin.

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Bemegride Antagonism to the Development of Physical Dependence on Barbitone in Rodents[1]

The problem of physical dependence on barbiturates and related sedative-hypnotics and minor tranquilizers is one of profound scientific and medical importance, especially in the present social atmosphere where misuse of drugs is so prevalent[2,3]. The present paper reports a study in the rat relating the intensity of phsyical dependence on the hypnotic barbitone to the depth of associated central nervous system (CNS) depression. The degree of CNS depression was modified by administering barbitone together with the analeptic bemergride[4].

Materials and methods. In 3 replicate experiments, groups of 20 female Wistar rats (140–180 g) received barbitone sodium (BARB), bemegride (4-methyl-4-ethylglutarimide, BEM) or a mixture of both drugs (BARB-BEM) by the regimen shown in Table I. The drugs were prepared in a saccharin solution (0.05%, SACC) to mask their taste and administered in the drinking water[5]. A control group of 20 rats received SACC alone (Table I).

Since the 3 drug solutions retained their potency for at least 24 h, they were changed each evening and the volume of fluid ingested by each group was determined. The behaviour of the rats was observed at various times throughout the day and each group was weighed twice

Table I. Oral dose regimen for rats

Drug[a]	Week of treatment				
(mg/kg/day)	1	2	3	4	5
BARB	100	200	300	400	500
BEM	75	100	125	150	150

[a] Given ad libitum in drinking water containing saccharin (0.05%).

[1] This work was presented in part by C.I.A. in November, 1973 in partial fulfilment for the degree of B.Sc. (Hons) in the University of Melbourne.
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weekly. The drugs were replaced by SACC on day 35 and the rats challenged 12, 24, 48, 72 and 96 h later with a minimally-convulsant dose of BEM (CD_1 = 10.9 mg/kg) given i.p. The number of animals in each group which showed spontaneous convulsions during the 96 h observation period or a tonic extensor seizure within 30 min of BEM challenge was noted.

Results and discussion. The following are the combined results of the 3 experiments. The average daily fluid intake of animals drinking the SACC, BARB, BEM and BARB-BEM solutions plateaued at 40, 15, 12 and 16 ml/rat respectively by day 10–15 and remained fairly constant thereafter. All groups gained weight at approximately the same rate until the 5th week when rats in the BARB and BEM groups lost an average of 10 and 20 g respectively. The average percentages of deaths in the groups receiving SACC, BARB, BEM and BARB-BEM were 0, 20, 12 and 16 respectively; deaths occurred sporadically in the BEM and BARB-BEM groups whereas most deaths in the BARB group occurred during the last 10 days.

Rats drinking BEM appeared slightly excited throughout the experiment but no tremors or convulsions were observed. Animals receiving BARB showed increasing CNS depression manifesting, at doses beyond 300 mg/kg, as weakness, ataxia and frequent loss of righting reflex. Rats receiving BARB-BEM were much less depressed than those given BARB alone and showed only mild ataxia at comparably high doses of BARB.

Following drug withdrawal on day 35, BARB-treated rats showed hyperexcitability for at least 48 h and 10% had spontaneous convulsions with a peak incidence at 24–48 h. Rats withdrawn from BARB-BEM were much less agitated while those withdrawn from BEM behaved normally; none of these animals convulsed spontaneously during the 96 h observation period.

The percentage of animals in each group which convulsed following BEM challenge is shown in Table II. None of the BEM-treated rats and only an occasional SACC-treated animal convulsed when challenged with BEM 12–96 h after drug withdrawal; indeed, the former animals showed very few signs of stimulation suggesting that tolerance to BEM may have developed. When similarly challenged, 50–70% of the BARB-treated rats had severe and long-lasting convulsions with a peak incidence at 48–72 h while only 10% of animals receiving BARB-BEM showed convulsions which generally were much briefer and milder.

Table II. Response to BEM challenge[a] following drug withdrawal

Time after withdrawal (h)	Percentage of rats convulsing[b]			
	SACC	BARB	BARB-BEM	BEM
12	0 (12)	12 (8)	0 (10)	0 (10)
24	8 (12)	50 (10)	10 (10)	0 (10)
48	16 (12)	70 (10)	9 (11)	0 (13)
72	0 (12)	60 (10)	10 (10)	0 (10)
96	0 (12)	50 (10)	10 (10)	0 (10)

[a] CD_1 = 10.9 mg/kg, i.p. [b] The number of rats challenged is given in brackets.

It appears then that the concomitant administration to the rat of the hypnotic BARB and the analeptic BEM decreased both the degree of CNS depression and the intensity of physical dependence produced by BARB alone. A similar result has been obtained in mice treated by the same drug regimen as the rat (Table I). However, conclusions were not as definitive in the mouse since the BEM-induced withdrawal syndrome was much less pronounced. It could be argued that the smaller number of rats in the BARB-BEM group responding to BEM challenge was due to the development of tolerance to BEM. However, this seems unlikely to be the primary cause since, following drug withdrawal, none of the rats in the BARB-BEM group showed spontaneous convulsions and all were much less agitated than rats which had received BARB alone. Notwithstanding, the development of tolerance to BEM in the rat is under investigation.

The present results suggest that BARB-induced physical dependence and CNS depression in the rat are related phenomena. It has been suggested that BEM analepsis to hypnotic depression occurs at certain synaptic sites in the ascending reticular formation, hypnotic and analeptic modifying there in opposite directions the release of transmitter substance presynaptically or the flux of ions postsynaptically, or both[6,7]. Since BEM attenuates both BARB-induced CNS depression and physical dependence, it is possible that these or associated synaptic reticular mechanisms are also important in the production of physical dependence on BARB and perhaps related CNS depressant drugs. The involvement of the reticular formation would be consistent with the widely-ranging signs and symptoms that comprise the abstinence syndrome following withdrawal of barbiturates in animals and man[2,3].

The present suggestions, if true, would lend support to theories on tolerance, physical dependence and the withdrawal syndrome based on the ability of certain CNS depressant drugs to promote presynaptic accumulation of transmitter substance at responsive central neurones[2,3,8]. A hypothesis has been proposed that attempts to explain for the barbiturates the occurrence of these phenomena in terms of the differential actions of the drugs on the functionally-interrelated protein and lipid components of the responsive synaptic membrane[4,7].

Zusammenfassung. Nachweis einer protektiven Wirkung bzw. Entwicklung einer Barbituratabhängigkeit durch die gleichzeitige Gabe des Analeptikums Bemegrid.

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[9] Acknowledgment. We gratefully acknowledge the generous gift of bemegride (Megimide) from Nicholas Pty Ltd, Australia.