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Biochemical Pharmacology, Vol. 21, pp. 3082–3085. Pergamon Press, 1972. Printed in Great Britain.

Inhibition of development of tolerance to morphine by cycloheximide

(*Received* 29 *April* 1972; *accepted* 19 *June* 1972)

Cochin and Kornetsky[1] have stated that tolerance to narcotic analgesics resembles, in some respects, an immune response. In ensuing years, these authors and others[2–4] have attempted to shed light on this resemblance. In considering this phenomenon, we felt that it might be profitable to investigate the effect on tolerance of agents affecting the immune response. One of the drugs we have studied is cycloheximide, which inhibits protein synthesis, and thus, RNA synthesis. We are reporting some of this work in this paper.

Way *et al.*[5–7] have shown that cycloheximide, given chronically to mice, inhibits the development of tolerance to an implanted pellet of morphine sulfate and reduces the intensity of physical dependence precipitated by an injection of naloxone. They attribute their results to effects on brain serotonin levels rather than on any possible immune response. Our work involved rats, and cycloheximide was given weekly rather than daily, because we wished to avoid the possibility of obtaining the desired effect (increased latency of reflex response time relative to control animals) by some nonspecific depression or debilitation of the animals. We observed at the start of our work that rats are far more sensitive to cycloheximide than are mice. The dose used chronically in mice (20 mg/kg) by Way *et al.*[5–7] is fatal to rats on the first administration.

Male rats weighing about 275 g were randomly assigned by weight into two groups, one receiving cycloheximide and morphine sulfate and one receiving only morphine sulfate. All rats were injected subcutaneously once a week for the first 13 weeks of the experiment, with the injections of cycloheximide (1 mg/kg) given 1 hr before the injections of morphine sulfate (10 mg/kg).

On alternate weeks from 1 to 13, the animals were tested for analgesia immediately after receiving their weekly dose of morphine, using the hot-plate method of Eddy and Leimbach;[8] areas under the time-effect curve were calculated using a modification of the method of Winter and Flataker.[9] At the end of this 13-week period, we lengthened the dosage interval in order to observe any possible differences in the rates of disappearance of tolerance in the two groups.

TABLE 1. EFFECT OF CYCLOHEXIMIDE (1 mg/kg, s.c.) ON THE ANALGESIC RESPONSE TO MORPHINE SULFATE (10 mg/kg, s.c.) IN THE RAT*

Treatment		1	3	5	7	9	13	17†	20
Cycloheximide plus morphine	Mean area (Raw ± S.D.)	2032‡	846	691	969	677	521	712	833
		817	386	476	781	557	281	653	781
	(Adjusted)		790	667	583	611	514	692	867
	N	22	22	21	17	15	11	11	6
	Av. wt.	320	366	418	437	477	506	575	589
Morphine	Mean area (Raw ± S.D.)	1535‡	646	232	140	196	208	217	375
		1062	652	279	180	481	125	215	521
	(Adjusted)		708	262	152	266	214	237	357
	N	20	20	17	14	11	11	11	11
	Av. wt.	322	389	442	476	499	528	570	580
Analysis of covariance	P		NS	$<0{\cdot}01$	$<0{\cdot}01$	NS	$<0{\cdot}01$	$<0{\cdot}05$	NS

* Areas under the time-effect curve are in minute-seconds. Weights are in g.
† Last dose of cycloheximide.
‡ Not significantly different, $P > 0{\cdot}05$.

The areas under the time-effect curve were examined using an analysis of covariance[10] with the analgesic response to the first dose of morphine or morphine plus cycloheximide as the covariate. There was no significant difference between the initial responses of the two groups. The results of a typical experiment are shown in Table 1. Several similar experiments have yielded essentially the same results: the two groups (cycloheximide and morphine-treated animals and those treated with morphine alone) became significantly different from each other at the fifth dose of drug. This was first noted in previous experiments in which the animals were tested at weekly intervals after every dose of morphine or morphine and cycloheximide.[11] In every experiment, the two groups remain significantly different from each other for several weeks. In the experiment reported here, the two groups are different on weeks 5 through 17, except for week 9. However, if the one animal whose response is out of line with its other data* is eliminated, the two groups are significantly different ($P < 0.05$).

The results after week 13, when the interval between doses was lengthened, show that the cycloheximide-treated animals return toward their original responsiveness to morphine more rapidly than do the control animals. If this is interpreted as a loss of tolerance to morphine, then it would appear that rats which are less tolerant to morphine may lose that tolerance more readily than rats which are more tolerant. This agrees well with the work of Miller and Cochin,[12] where mice were injected daily with either 6 or 8 mg/kg of morphine sulfate. The 6 mg/kg group developed less tolerance to morphine, showing a greater analgesic response than the 8 mg/kg group to the fourth injection. This group also returned to its original sensitivity to morphine after a drug-free interval of 37 days, while the 8 mg/kg group did not. Both sets of experimental results suggest that sensitivity to morphine is regained more easily on discontinuance of drug administration when the tolerance developed is less profound.

These results suggest that cycloheximide alters the rate of development of tolerance to morphine sulfate in the rat. Both groups of animals (morphine alone and morphine plus cycloheximide) exhibit the expected development of tolerance to the analgesic effect of morphine in response to the first few doses of morphine. However, as may be seen in Table 1, the analgesic response of the control group continues to fall to a mean adjusted area of about 250 min–sec, while the analgesic response of the cycloheximide-treated group remains at about 600 min–sec. Thus, cycloheximide seems to prevent the full development of tolerance.

Additional experiments were performed to investigate possible analgesic effects of cycloheximide. A group of rats were given cycloheximide (1·5 mg/kg, s.c.) weekly, and tested biweekly for analgesia on the hotplate. No morphine was given. These tests yielded areas under the time-effect curve that did not differ significantly from those of saline-treated animals.

The experiments described above offer support to the hypothesis that tolerance to narcotic analgesics is a phenomenon involving the synthesis of one or more proteins. Cycloheximide in the doses given does not cause weight loss, although the animals did not gain weight as rapidly as did controls. The animals appear normal, save for some hair loss, which is seen only after 20 or more injections of cycloheximide. Thus, the effect does not seem one of debilitation caused by cycloheximide.

These results agree with those of Way *et al.*[5] obtained in experiments in which cycloheximide was given daily to mice. Our results are also consistent with the enzyme de-repression theories of Goldstein and Goldstein[13] and of Shuster,[14] and with the enzyme stabilization theory of Goldstein and Goldstein,[15] as well as with the hypothesis of Collier,[16] which postulates an increase in secondary receptors.

All of the hypotheses cited propose the synthesis of protein, either a receptor protein, an enzyme, or a possible circulating protein. The alteration of the rate of development of tolerance to morphine by cycloheximide is consistent with such an inhibition of protein synthesis.

Acknowledgements—We wish to thank Mrs. R. Grell for her technical assistance. Cycloheximide (Acti-dione) was donated by The Upjohn Company, Kalamazoo, Mich.

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* Animal 440 (Control)

Week	1	3	5	7	9	13
Area	3420	828	120	36	1736	298

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Biochemical Pharmacology, Vol. 21, pp. 3085–3088. Pergamon Press, 1972. Printed in Great Britain.

Effect of 6-hydroxydopamine on the activity and circadian rhythmicity of hepatic tyrosine aminotransferase

(*Received* 21 *April* 1972; *accepted* 27 *June* 1972)

THE INDUCIBLE enzyme,[1] tyrosine aminotransferase (ECC 2.6.1.5), undergoes circadian variations in activity,[2] and it has been proposed that these variations are under noradrenergic control. According to this proposal, aminotransferase synthesis is cofactor dependent and norepinephrine, periodically released from noradrenergic terminals, disrupts synthesis by directly chelating the cofactor, pyridoxal phosphate, and removing it from the enzyme-forming site.[3] In support of this proposal it has been established that norepinephrine, together with other catechols, does complex with pyridoxal-5-phosphate *in vitro*[4,5] and that the dissociation constant for the resulting isotetraquinoline is smaller than that of the enzyme–cofactor dissociation constant. Further, administration of pyridoxine to the rat can lead to elevations in tyrosine aminotransferase activity under some conditions,[6] and norepinephrine can inhibit this elevation.[7] This inhibition is most marked if norepinephrine is administered during the nocturnal rise of enzymic activity.

In the present study we attempted to gain more information on the relationship betwen norepinephrine and enzymic activity by examining circadian changes in enzymic activity after treatment with 6-hydroxydopamine, an agent shown to be a potent tool for selective destruction of catecholaminergic terminals, particularly noradrenergic terminals.[8]

Male Long–Evans rats weighing 80–120 g were housed individually after treatment and maintained on an *ad lib.* diet of Purina Rat Chow. The animal room was maintained at 22° and lights were on from 5 a.m. to 5 p.m. Animals were decapitated at 9 a.m. or 9 p.m. which are the nadir and peak, respectively, of the diurnal rhythm of tyrosine aminotransferase under these environmental conditions. Upon decapitation, trunk blood was collected; organs were quickly removed, rinsed, weighed and placed in appropriate media for assay. Spleens and hearts from some animals were homogenized in 0·4 N perchloric acid for norepinephrine determinations. Livers were homogenized in cold neutral 0·15 M KCl and centrifuged at 105,000 *g* for 30 min at 4° and the supernatants used for liver tyrosine aminotransferase assay. Adrenal and serum corticosterone was assayed by the method of Givner and Rochefort.[9] Norepinephrine was assayed by the method of Bertler *et al.*[10] after separation by the method of Anton and Sayre.[11] Liver tyrosine aminotransferase was measured by the method of Lin *et al.*[12] and protein by the method of Lowry as described by Layne.[13] A Student two-tailed *t*-test was used for statistical comparisons.