In a group of 7 experiments, 10 min after the last dose-response curve for norepinephrine, one half of the atrium received a dose of tyramine (10^{-4}) and 5 min later (without previous washing) both were exposed to phenoxybenzamine (10^{-6}, for 20 min). Dose-response curves for norepinephrine were repeated 20, 50, 80 and 110 min later in both halves. Figure 1 illustrates the results of a typical experiment. It can be seen that the irreversible increase in sensitivity to norepinephrine (Figure 1-A) which appears after phenoxybenzamine treatment, is inhibited (Figure 1-B) when the atrium was first exposed to tyramine. In another set of 8 similar experiments desmethylimipramine was used instead of phenoxybenzamine. After initial dose-response curves for norepinephrine were determined, one half of the atrium received tyramine (10^{-4}) and 5 min later both were exposed to desmethylimipramine (10^{-6}, for 20 min). Sensitivity to norepinephrine was again tested 20, 50, 80 and 110 min later. Figure 2 shows the results of a typical experiment. It is interesting to observe that, in this particular case, no difference in sensitivity to norepinephrine was found between the control (Figure 2 A) and tyramine-treated preparation (Figure 2 B), when they were exposed to desmethylimipramine.

Discussion. It is well established that phenoxybenzamine and desmethylimipramine potentiate the responses to norepinephrine. This potentiating effect is believed to be due to the ability of both, phenoxybenzamine and desmethylimipramine, to inhibit the uptake of norepinephrine into the nerve terminals.

The results of the present investigation show that the potentiation of the responses to norepinephrine induced by phenoxybenzamine, can be prevented by the presence of tyramine during the period of exposure to phenoxybenzamine. These findings provides further evidence for a common uptake site for norepinephrine and tyramine on adrenergic nerve terminals. On the other hand, our results show that tyramine, when present during exposure to desmethylimipramine, was not capable of preventing the potentiation of norepinephrine responses induced by this blocking agent.

Provided that both, phenoxybenzamine and desmethylimipramine, share the capacity to block the uptake of norepinephrine by adrenergic neurones, the fact that tyramine was able to prevent the uptake blocking action of phenoxybenzamine but not that of desmethylimipramine, can be taken as an evidence for a different site of action on catecholamine uptake system.

Resumen. El tratamiento con fenoxibenzamina o desmetilimipramina da lugar a una potenciación irreversible de las respuestas a noradrenalina, en la aurícula aislada de cobayo. La presencia de tiramina durante el tratamiento previene dicha potenciación cuando se utiliza fenoxibenzamina, mientras que no la modifica en el caso de la desmetilimipramina.

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Effect of Constant Lighting on the Morphine Susceptibility Rhythm

Morphine sulfate displays quantitative variations of susceptibility along a 24-hour time scale characterized by peak analgesia (i.e. crest) during the dark and a trough in the light period[1]. Reversal of the lighting regimen produced an inversion of the morphine susceptibility pattern suggesting a prevalent photoperiodic effect on morphine susceptibility[2]. Investigators have reported that pentobarbitol susceptibility was suppressed by programming mice in continuous darkness[3]. Similarly, pineal epinephrine rhythms have been abolished in rats maintained in continuous darkness or blinded by bilateral orbital enucleation[4]. Some 24-hour rhythms have been suppressed or abolished by constant lighting[5,6]. A continuous lighting schedule effect is reported herein as further evidence of the dominant role of photoperiodicity on the susceptibility pattern of morphine analgesia.

Adult female albino CF-1 mice (25.4 ± 0.4 g body wt.) were housed and tested with conditions, procedures and morphine dosage as previously reported[1,2], except for the constant lighting schedule and incrased body weight of about 2 g due to aging over the 4 week test period. An initial standard light period (I) had lights on 06.05–18.05 h and total darkness from 18.05–06.05 h[1]; a second experiment (II) had the lighting regimen reversed[2] and in this study (III) the mice were exposed to continuous light for a period of 14 days prior to the morphine administration. A standardization time of 14 days is considered adequate to facilitate resynchronization[7].

Beginning at 12.00 h and at 3-hour intervals over the succeeding 24-hour period, groups of 20 mice were pretested, weighed and injected i.p. with morphine sulfate, 8 mg/kg. Exactly 20 min after the morphine injection each mouse was retested for the presence of pain as determined by a modified Haffner tail pinch clamp method[8]. All mice failing to display a positive pain response (i.e. biting clip) within 30 sec were considered to be analgesic[8]. Each analgesic response was converted to per cent analgesia relative to the total number of mice

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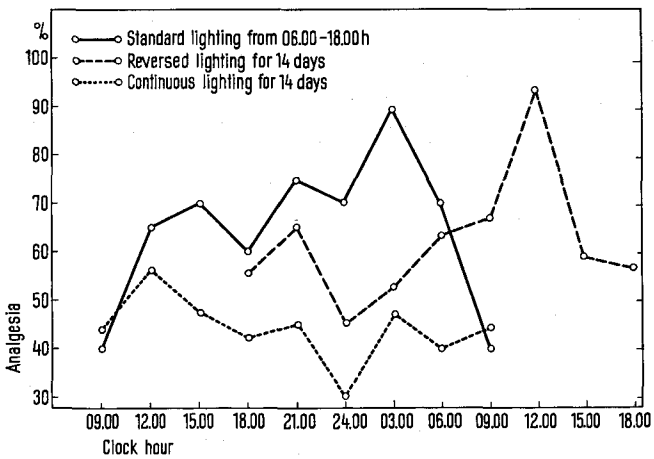

Mean 24-h morphine analgesia patterns. ○—○, standard light from 06.05 h to 18.05 h; ○---○, reversed lighting for 14 days; ○...○, continous lighting for 14 days.

injected at that time period. A mean 24-hour morphine analgesia response was determined by pooling the analgesic responses from each time period. The statistical methods of SNEDECOR[9] were programmed and performed on Wang 700 A.

Experiment I showed that, under standard lighting, peak analgesia occurred at 03.00 h (dark period) with a trough at 09.00 h (light period)[1]. The same mice placed in a reversed light schedule (II) for 14 days prior to morphine administration displayed a temporal 180 degree shift in that there then was a peak at 12.00 h (also dark period) and a trough at 24.00 h (light period)[2]. The present experiment (III) using the same mice, but now housed in continuous light for 14 days prior to morphine administration, shows that the analgesic rhythm has been suppressed (Figure). No peak is evident and there is a small but insignificant trough. Conversely, the standard (I) and reversal (II) experiments showed statistically significant differences between the trough and peak times[1,2].

Suppression of 24-hour variations suggest that the rhythmical analgesia pattern can be manipulated by 24-hour changes in environmental lighting and that light could be a dominant synchronizer of the organism's circadian system to morphine susceptibility. Under constant lighting conditions, some rhythmic functions have been shown to ‚free run' (i.e. the period lengths deviate from 24-hours depending on the species)[10]. The peak of the particular rhythmic function may occur at different times every day. In study III, neither peak nor trough was evident.

It would appear as if the analgesic pattern to morphine is dependent on the lighting regimen. In alternating the light-dark cycles of 12-hours each, or a reversal of this schedule, peak analgesia is always in the dark period and the trough always in the light period. In continuous light the pattern is lost; that is, there are no statistically significant differences between percent analgesia at 09.00 h and 21.00 h, or 15.00 h and 03.00 h, or even the extremes of 12.00 h and 24.00 h. There were no differences between the 24-hour mean analgesia responses for I and II, although on each experimental day there was a significant difference between the light and dark phase responses[2]. An analysis of variance of mean percent analgesia data from experiments I, II and III revealed a significant difference between the 3 experimental days (i.e. p 0.01–0.001). A Chi-square test of the pooled mean percent analgesia data from I and II versus III revealed a highly significant greater analgesia ($p < 0.001$) in experiments I and II than in experiment III. Such a difference might be due to the fact that the continous light regimen had reduced the 24-hour mean response for III since analgesia responses are less in the light than the dark phases[1,2].

To test this possiblity a Chi-square analysis of the light phase data for pooled experiments I and II versus experiment III was performed. A highly significant difference remains (p 0.004) even with the dark phase data omitted from experiments I and II. Consequently, the presence of light phase data only during III would not appear to be the determining factor for the reduced analgesic activity of morphine in III. A second possiblity could be the occurrence of a lunar phenomenon noted in an earlier work[1]; that is that a greater degree of analgesia occurs during full moon than during new moon. Testing for lunar cycles but only with the light phase data, that is full moon (I and II) versus new moon (III) revealed a significant greater degree of analgesia during full moon than new moon (p 0.017). Pooling the earlier lunar data[1] and this new data and then retesting by Chi-square showed a highly significant difference between new and full moons (p 0.004). It would therefore appear as if there might well be a lunar as well as circadian rhythm to morphine susceptibility.

The studies described here indicated that photic information is one exogenous force (i.e. dominant synchronizer) controlling the morphine susceptibility pattern. This study supports the role of the lighting regimen in drug rhythmicity. Hence, the extrinsic lighting factor should be considered as an important variable in determining the efficacy of an analgesic dose of morphine sulfate administered throughout a 24-hour or a 28-day period.

Zusammenfassung. Durch Tag-Nacht-Umkehr wird bei Mäusen eine Verschiebung der Sensibilität für die schmerzstillende Wirkung des Morphins erzielt, aber dem ständigen Licht für 14 Tage ausgesetzt, verlieren sie die Sensibilität.

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