Circannual variation in sensitivity of the guinea-pig isolated ileum to naloxone

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It is well known that responses to drugs may vary in a rhythmic manner as a function of time. Most studies have been concerned with the variations found over 24 h, however, and there are few reports on seasonal or circannual (12 month) rhythms in drug susceptibility (Reinberg & Halberg 1971; Scheving et al 1974).

It has been demonstrated that segments of intestine taken from chronically morphinized guinea-pigs (Ehrenpreis et al 1972; Villarreal & Dummer 1973; Schulz & Herz 1976; Rodríguez et al 1978) as well as ilea incubated with morphine (Villarreal et al 1977; Luján & Rodríguez 1978) respond with strong contractions to the in vitro administration of naloxone. While attempting to pharmacologically characterize this phenomenon, we observed a striking correlation between the responsiveness of the tissue to the antagonist and the time of year in which the experiments were performed. Ilea taken from animals during the summer (June–July) were much more sensitive to naloxone than those removed and tested during the winter (December–January). It seemed worthwhile to determine whether this variation in responsiveness followed a regular rhythm, using data that had been obtained over the past two years.

Experiments were carried out daily, Monday through Friday, from January, 1977 to December, 1978, with adult male guinea-pigs, 300–600 g, housed in metal cages in the laboratory, exposed to the seasonal influences of natural light, for at least 5 days after receipt from the supplier before use. Commercial chow, alfalfa and water were freely available.

The animals were killed between 0900–1000 h by a sharp blow on the head, and the small intestine was rapidly removed; the terminal section was used after the 10 cm nearest to the ileo-caecal junction had been discarded. Segments of 1·5 cm were set up in a 10 ml bath with Krebs-bicarbonate solution (KB) of the following composition (mM): NaCl, 118·0; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 25·0; glucose, 11·0; choline chloride, 0·03, which had been warmed to 37°C and bubbled with 5% CO_2 in oxygen. The resting tension was fixed at 1 g and the longitudinal contractions (tension) of the segments were recorded isometrically through a Grass FT-03 force displacement transducer on a polygraph. Tissues were allowed to reach equilibrium for 30 min before being superfused with prewarmed aerated KB containing morphine (1×10^{-6} M) at a rate of 10 ml min^{-1} for 240 min. Control segments were superfused with KB alone. Naloxone was then added to the bath, immediately after the perfusion was stopped. The peak tension was determined in order to quantitatively evaluate the contractile response. Each segment was used for only one determination.

As reported elsewhere (Luján & Rodríguez 1978), segments of intestine incubated for 4 h with morphine at 37°C undergo an intense dose-dependent contraction following naloxone administration in vitro. The response is similar in magnitude and form to that produced by the antagonist in ilea taken from guinea-pigs morphinized in vivo (Rodríguez et al 1978). Villarreal et al (1977) have provided evidence that further supports the qualitative and quantitative isomorphism of narcotic dependence and precipitated abstinence in vitro, and their counterparts in vivo. Our present results show that the guinea-pig isolated ileum displays a pronounced rhythmic variation in sensitivity to the spasmogenic action of naloxone over a 12 month cycle. The variation in responsiveness of ilea preincubated with morphine to naloxone ($3{\cdot}84 \times 10^{-6}$ M) during the 2 year observation period can be seen in Fig. 1 (panels A, B). The mean weekly values for naloxone-induced contractions progressively increase from the first weeks of each year, reach a maximum at about the 26th week, and then slowly decline to a minimum in the last 4 weeks. The peak values during June–July are 4–5 times greater than those in the winter months.

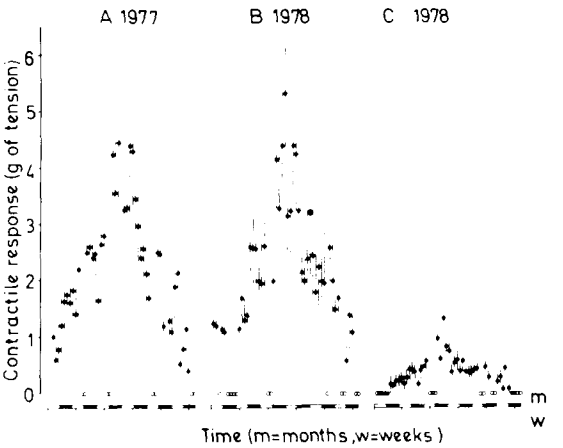

FIG. 1. Changes in responsiveness of the guinea-pig isolated ileum to naloxone ($3{\cdot}84 \times 10^{-6}$ M) during the successive weeks of 1977 and 1978. Mean ($n = 5$–10) weekly values with s.d. are shown for naloxone-induced contractions in ilea preincubated with (panels A, B) and without (panel C) morphine (1×10^{-6} M) for 240 min. Only data for 1978 were available for the latter. Open circles indicate weeks for which data was not recorded.

* Correspondence.

An attempt was made to analyse the apparent rhythmicity of this phenomenon using the periodogram method (Enright 1965). A total of 104 mean weekly values for naloxone-induced contractions in segments exposed to morphine were first smoothed by the Hanning filter procedure (Blackman & Tukey 1958). Values for the weeks in which data were unavailable were considered equal to 0. The results of the periodogram analysis were further processed to eliminate an increasing linear trend (Bendat & Piersol 1971) due to the small number of complete cycles.

The primary feature of the periodogram that was obtained is an amplitude peak at or close to 52 weeks (Fig. 2). The estimated period for the dominant component, based on an interpolation between Ap (frequency estimate) values (Enright 1965), is 51·95 weeks. This analysis demonstrates a strong circannual rhythm in the responsiveness of morphinized ileum to a fixed dose of naloxone. An independent estimate of the period of overt rhythmicity was obtained by using a mixed-radix fast Fourier transform (FFT) algorithm (Singleton 1969) to determine a power spectrum (relative content of each frequency component within the overall cyclic pattern). The estimated period was equal to 52 weeks, in agreement with that obtained from the periodogram analysis.

Since variations in the height of tissue responses to a fixed dose of a drug do not necessarily indicate a change in sensitivity, we analysed all the dose-response curves obtained during 1978 for naloxone-induced contractions in ilea preincubated with morphine. The data presented in Fig. 3 represent experiments performed on different days within each month. The sensitivity of the tissue to the spasmogenic effects of naloxone is about 10–100 times greater in June and July than during other months of the year. Doses of naloxone higher than $3{\cdot}84 \times 10^{-6}$ M did not increase, and in some cases diminished, the magnitude of the response.

These results prompted us to examine whether the effect of the antagonist in control segments also varied circannually. Previously, naloxone was found to produce modest but dose-dependent contractions in segments not exposed to morphine (Rodríguez et al 1978). Reviewing data accumulated during 1978, it was ascertained that their pattern of responsiveness is parallel to that observed in morphine pretreated ilea (Fig. 1, panel C) and that the period estimate is also 52 weeks (FFT). During December and January, the control intestines were completely insensitive to naloxone, even at doses as high as $7{\cdot}68 \times 10^{-6}$ M. This fact, and the low sensitivity of the tissues observed during autumn and spring, might explain the failure of other workers (Frederickson et al 1976; Schulz & Herz 1976) to record a response to naloxone in ilea not exposed to morphine.

Long-term fluctuations in pharmacological effects have been demonstrated for some drugs (Reinberg & Halberg 1971), including morphine and naloxone. For example, Davis & Khalsa (1971) observed that naloxone-induced aggression associated with withdrawal in morphine-dependent rats occurred more frequently in

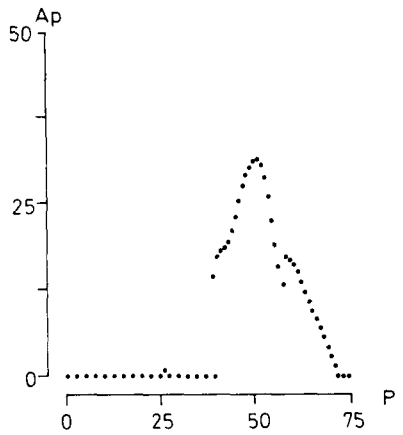

FIG. 2. Periodogram obtained from an analysis of 104 mean weekly values of contractile responses to naloxone ($3{\cdot}84 \times 10^{-6}$ M) in ilea preincubated with morphine. The amplitude of the frequency estimate (Ap) is plotted against presumed periods (P). Only data for 75 weeks are plotted. The corresponding period for the primary peak is equal to 51·95 weeks.

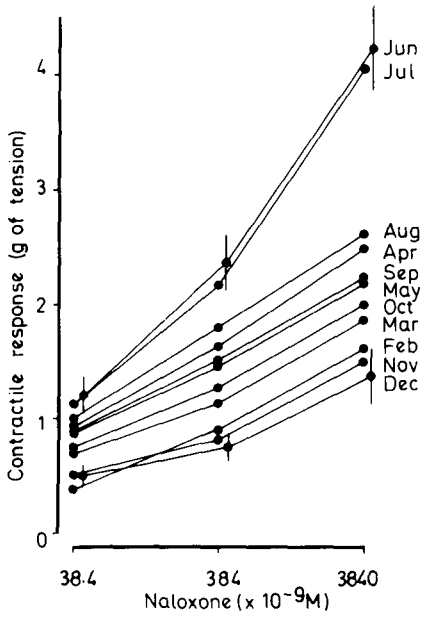

FIG. 3. Dose-response curves for naloxone-induced contractions in ilea preincubated with morphine (1×10^{-6} M) for 240 min. Each point represents the mean of at least 8 experiments performed during the indicated month. Vertical bars show the representative s.e. for certain months. The curve for January is omitted due to the incompleteness of the data.

the summer than in the winter, and Bowman & Buwembo (1972) found that morphine is more effective in inhibiting electrically-induced contractions in the chick isolated oesophagus in summer than in winter. Of particular interest is the seasonal variation reported by Shoham-Moshonov & Weinstock (1977) in a careful and quantitative study on the development of tolerance to the effects of morphine by coaxially-stimulated guinea-pig isolated ileum. A significantly greater number of ilea developed tolerance during May–November than during December–April. These experimental observations are consistent with the view that biological systems are more sensitive to the effects of morphine in summer than at any other time of the year.

The contractile response of morphinized ilea to naloxone appears to be mediated through the release of acetylcholine (ACh), since it is blocked by atropine (Ehrenpreis et al 1972). This fact suggests that variations in sensitivity to naloxone could be explained by parallel changes in the sensitivity of smooth muscle cells to ACh. However, experiments designed to investigate this possibility have failed to support this line of reasoning. The sensitivity of longitudinal muscle to ACh is 2–3 times lower in June–August than during November–February, as shown by a shift to the right of the dose-response curve. A similar observation was previously reported by Weinstock & Shoham (1974), who also found that ACh release from stimulated ilea is higher in summer than in winter (Shoham-Moshonov & Weinstock 1977), in agreement with Hazra (1975). Moreover, there is evidence that the release of ACh from other tissues varies with the seasons as well, and is highest in summer (Monnier & Herkert 1972). Thus, the variation in sensitivity to naloxone appears to be related more to changes in ACh release from nerve endings than to tissue reactivity.

It is feasible that the circannual rhythm is associated with cyclic changes in acetylcholinesterase activity, as seasonal variations in enzymatic activity are not uncommon (Kennedy & Nayler 1965; Nayler 1968; Beuthin & Bousquet 1970). The finding of Monnier & Herkert (1972) that the concentration of ACh in the haemodialysate of rabbits is much lower in winter than in summer suggests such a possibility.

Another factor to be considered is the presence of peptides with narcotic-like actions in the myenteric plexus of the guinea-pig ileum (Smith et al 1976). It has been shown that the sensitivity of this tissue to naloxone is time-dependent and is related to the opiate concentration (Luján & Rodríguez 1978). Hence, it is conceivable that oscillations in sensitivity to the antagonist might be due to rhythmic fluctuations in the concentration of endorphins in the tissue. This remains to be confirmed.

Whatever the underlying mechanisms might be, the present analysis clearly demonstrates a circannual variation in the sensitivity of guinea-pig ileum to naloxone. The presence of a peak in the sensitivity of the tissue to the spasmogenic action of the antagonist at the beginning of the summer was found in both morphine-exposed and unpretreated ilea. We feel that these observations have important implications for the design of studies aimed at the elucidation of the mechanisms of narcotic action, and will permit opioid effects to be measured with greater accuracy.

November 30, 1979

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