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ACUTE TOLERANCE TO NARCOTIC ANALGESIC DRUGS IN RATS

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It has been reported that treatment of rats and mice with actinomycin D, an antibiotic inhibitor of protein synthesis, diminishes the impairment of the analgesic effect of morphine normally observed during chronic treatment with the analgesic (Cohen, Keats, Krivoy & Ungar, 1965). The widespread metabolic disturbances that arise during prolonged treatment with inhibitors of protein synthesis (Young, Robinson & Sacktor, 1963) make it difficult to interpret this result, and in fact, some of the animals receiving higher doses of actinomycin died.

In the experiments reported in the present paper it is shown that in rats, tolerance to morphine and other analgesics given by continuous intravenous infusion develops within 4 hr and this makes it possible to study the effects of actinomycin D on tolerance while overall metabolism is apparently unimpaired.

METHODS

Preparation of animals for intravenous infusion

Male or female Wistar rats weighing between 130 and 250 g were anaesthetized with ether. A polyethylene cannula filled with saline was inserted into a jugular vein and the free end was exteriorized through a stab wound in the skin at the back of the neck. On the next day, solutions of drugs (details in RESULTS) dissolved in 0.9% (w/v) sodium chloride solution were infused into the conscious animals from a continuous infusion apparatus connected to the jugular vein cannulae by fine polyethylene tubes. The rate of infusion was 1 ml./hr.

Measurement of analgesia

Analgesia was measured by a modification of the method of Green & Young (1951). A force was applied to the tip of a rat's tail by the plunger of a syringe connected to a compressed gas supply (Morton, 1962). Pressure developed within the syringe (which was measured by a U-tube mercury manometer) created the force that was exerted by the plunger on the rat tail. The rate of rise of pressure in the syringe was adjusted to 2 cm. Hg/sec.

Before the infusion was started and at various times during infusion, the threshold pressure at which the rat responded to the force applied to the tail was measured. The threshold was taken as the pressure at which the rat first responded by struggling or squeaking. The pressure within the syringe was never increased above 32 cm Hg, which was about six times the mean initial threshold pressure.

Each measurement was expressed as an "index of analgesia," calculated by $\Delta obs/\Delta max$, where $\Delta obs = P_{obs} - P_i$ and $\Delta max = P_{max} - P_i$. ($P_i$, P_{obs} and P_{max} are the pressure thresholds recorded before

drug treatment, at times after drug administration, and the maximum pressure, respectively.) P_{max} was taken arbitrarily at 36 cm Hg, but in practice pressure stimuli greater than 32 cm Hg were never used. For example, where an animal with $P_i=6$ responded to a pressure of 32 cm Hg, the analgesic index would be $\frac{32-6}{36-6}=0.87$; where such an animal did not respond at 32 cm Hg the index was taken as 1.00.

Motor co-ordination test

The time during which rats (tested individually) were able to balance on a rotating horizontal rod was measured. The P.V.C. covered rod (diameter 25 mm.) was rotating at 1.1 rev/min.

Nitrogen excretion

Measurements of total nitrogen content of urine were made by Kjeldahl's method.

The following drugs were used: morphine HCl, diamorphine HCl, pethidine HCl, etorphine HCl, actinomycin D (Dactinomycin, Merck, Sharp and Dohme).

RESULTS

Preliminary experiments were carried out to discover a suitable range of infusion rates of morphine into conscious rats. The criteria chosen were such that, at least initially, significant levels of analgesia should be produced and that the rates of infusion should

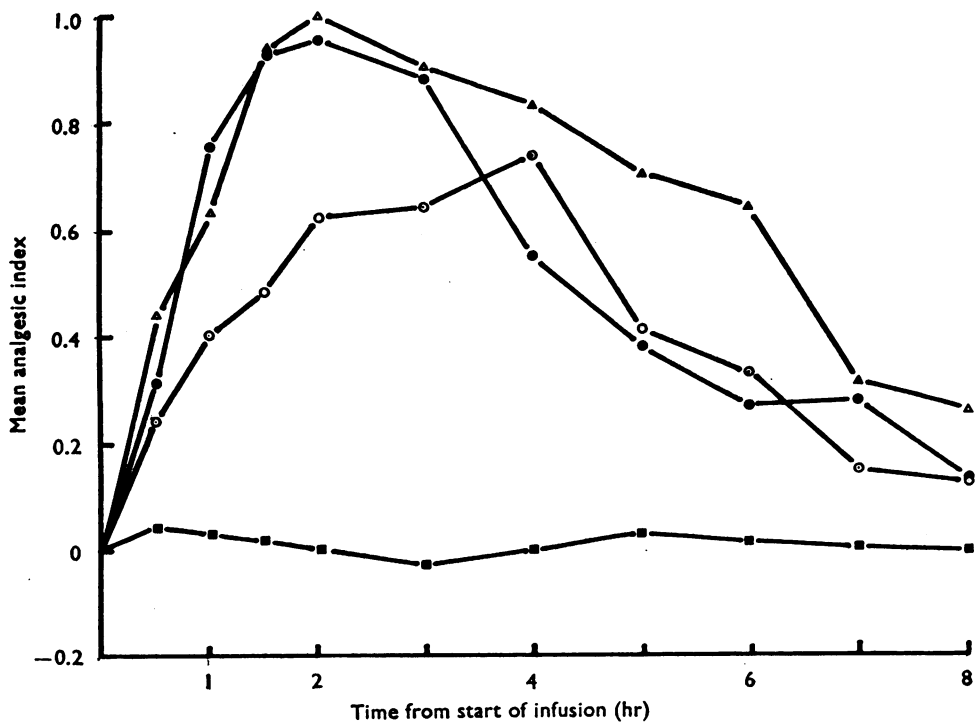

Fig. 1. Mean analgesic indices during intravenous infusions of morphine. ○, Morphine 5 mg/kg/hr ($n=4$); ●, morphine 7.5 mg/kg/hr ($n=7$); △, morphine 10 mg/kg/hr ($n=5$); ■, 0.9% sodium chloride solution ($n=4$).

be much slower than those that might be lethal during continuous administration over periods up to 8 hr. Morphine infusion at rates of 5, 7.5 and 10 mg/kg/hr were found to satisfy these criteria. Figure 1 shows the mean analgesic indices measured at 30 and then 60 min intervals in rats receiving morphine at these rates over a period of 8 hr, and in control experiments in which the rats were infused with 0.9% (w/v) sodium chloride solution only.

Three phases in the analgesic response to morphine can be discerned. These are, first, a phase of increasing analgesia 90–120 min in duration, followed by a period in which peak analgesia was maintained for a further 90–120 min, when with the higher rates of infusion most animals (ten out of twelve) did not respond to a pressure stimulus of 32 cm Hg. Thereafter the animals gradually became more responsive to pressure stimulation until after 8 hr of infusion all animals responded to pressures of less than 17 cm Hg. The peak values for the analgesic indices were between four and eight times greater than those measured after 8 hr of infusion. During the phase of peak analgesia the animals showed little voluntary activity and were markedly catatonic, but as the analgesia declined they became more active and the catatonia was less pronounced. In the group receiving morphine at the rate of 7.5 mg/kg/hr, at peak analgesia six out of seven animals did not respond to the maximum stimulus, and this infusion rate was used in all subsequent experiments with morphine.

It seemed possible that the phase of declining analgesia might be the result of damage to the tail caused by the repeated application of pressure. Alternatively the animal might have become accustomed to the test procedure after repeated testing and so conditioned to display the motor reactions in response to aspects of the procedure apart from the

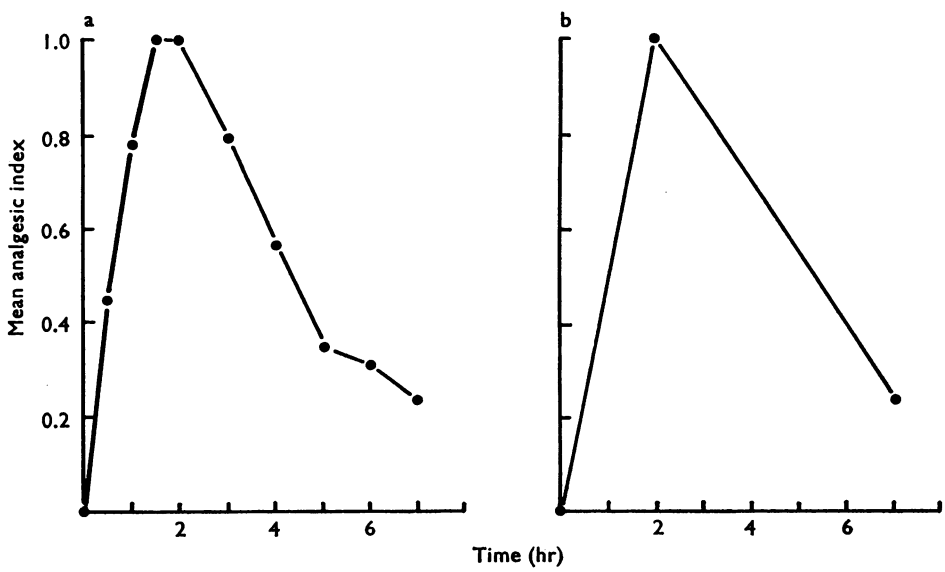

Fig. 2. Effect of varying the number of applications of the pressure stimulus on the analgesic response to an infusion of morphine. Morphine was infused at a rate of 7.5 mg/kg/hr. a: Results obtained from animals subjected to the pressure stimulus on ten occasions; b: results from animals tested on three occasions only. Five animals in each group. There was no significant difference between the two treatments.

pressure stimulus. Figure 2 shows the results of an experiment in which these possibilities were investigated. Rats, in two groups of five animals, were given infusions of morphine. In one group analgesia was tested at 30 min intervals for the first 2 hr, and at hourly intervals thereafter. The pressure thresholds of the second group were determined before the start of the infusions and at 2 and 7 hr only, while at intermediate times they were placed with the tail under the syringe plunger but no pressure was applied. There was no significant difference between the results given by the two groups of animals. It therefore seems unlikely that tail damage or conditioning to the test procedure can account for the fall in the level of analgesia.

Three other narcotic analgesics—diamorphine, etorphine and pethidine—have also been infused intravenously into rats (Fig. 3). In experiments with diamorphine and etorphine the course of the analgesia was similar in pattern to that found in experiments with morphine. During experiments in which pethidine was infused at 10, 20 or 30 mg/kg/hr the phase of increasing analgesia was more prolonged than with the other drugs.

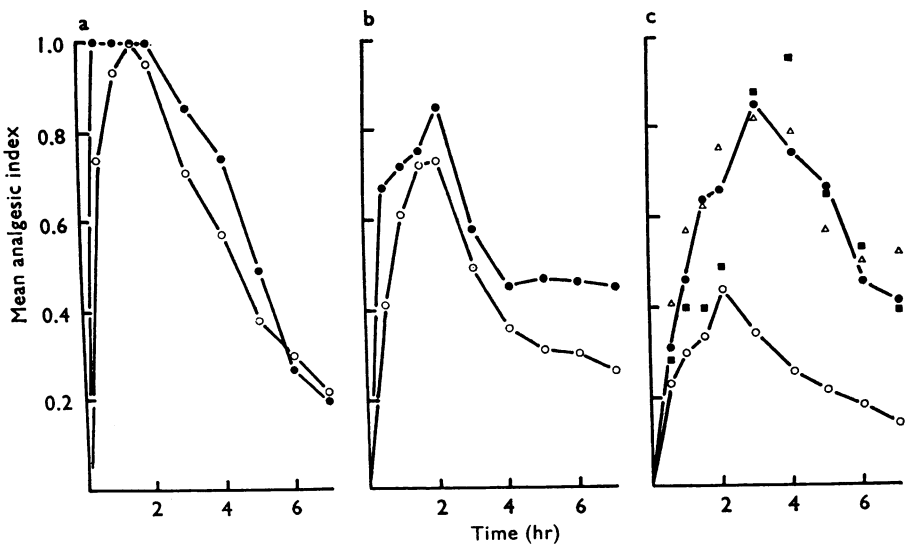

Fig. 3. Mean analgesic indices during infusions of diamorphine, etorphine and pethidine. a: Diamorphine; ○, 1 mg/kg/hr (n=5); ●, 2 mg/kg/hr (n=4). b: Etorphine; ○, 0.004 mg/kg/hr (n=6); ●, 0.008 mg/kg/hr (n=3). c: Pethidine; ○, 5 mg/kg/hr (n=4); ●, 10 mg/kg/hr (n=4); △, 20 mg/kg/hr (n=3); ■, 30 mg/kg/hr (n=4).

Effect of actinomycin D on the course of analgesia during infusions of analgesic drugs

Figure 4 shows the results of experiments in which analgesia was measured in rats receiving infusions of morphine with or without the addition of actinomycin D to the infused solution. (Actinomycin D was infused at a rate of 10 μg/kg/hr.) In animals receiving morphine alone the course of the analgesia was typical with the three distinct phases. After 7 hr ten out of eleven animals were responding to pressures less than 18 cm Hg (mean pressure threshold 14 cm Hg) although most of them (nine out of eleven) did not respond to 32 cm Hg pressure during the peak analgesic phase. In contrast, the

phase of declining analgesia was delayed or abolished in animals receiving morphine together with actinomycin D so that five out of eight animals remained unresponsive to the stimulus of 32 cm Hg pressure, after 7 hr of infusion. The remaining three animals required pressures of 23 cm Hg or more to induce a response. Actinomycin D also delayed the phase of declining analgesia when infused together with diamorphine, etorphine or pethidine (Fig. 5).

An attempt was made to estimate the duration of action of actinomycin D by giving it together with morphine for the first 4 hr of an infusion period, but thereafter giving morphine only (Fig. 6). The results obtained from animals given this treatment (C) were compared with those obtained on the same occasion from animals receiving either morphine only (treatment A) or morphine plus actinomycin D throughout the infusion (treatment B). Within 1 hr of the cessation of actinomycin treatment there was a clear difference between the level of analgesia in these animals (C) and those which continued to receive actinomycin (B). Thereafter tolerance continued to develop in the C group of animals at approximately the same rate as in the animals receiving morphine alone (treatment A). Thus the effects of actinomycin D ceased to be significant between 30 and 60 min after the termination of a 4-hour infusion.

The possibility that actinomycin D affected the rate of onset of analgesia was studied. Pethidine was used in these experiments because the rate of onset of analgesia was slower

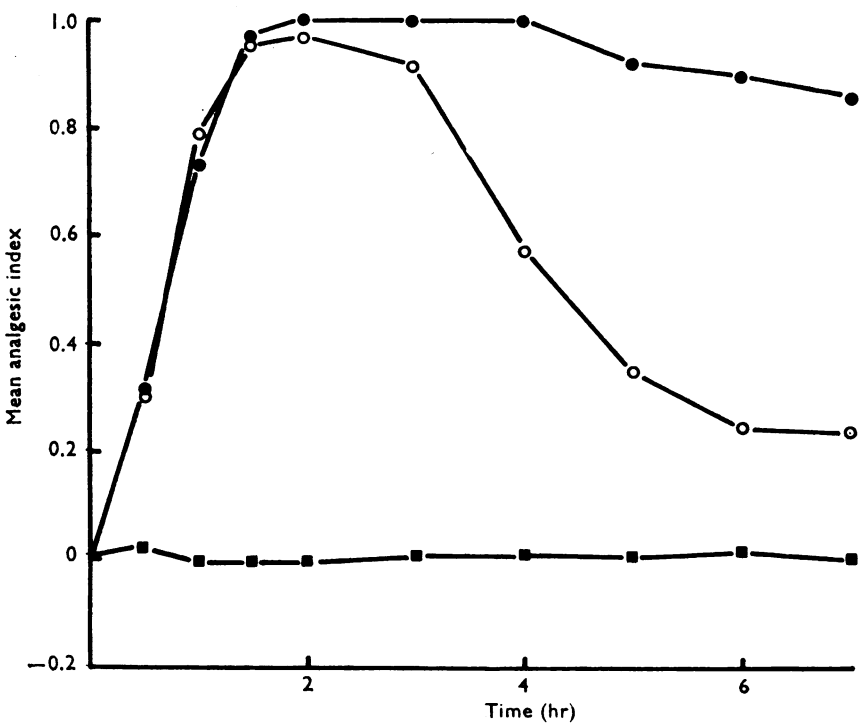

Fig. 4. Effect of actinomycin D on the analgesic response to an infusion of morphine. ○, Morphine 7.5 mg/kg/hr ($n=11$); ●, morphine 7.5 mg/kg/hr plus actinomycin D 10 μg/kg/hr ($n=8$); ■, actinomycin D 10 μg/kg/hr ($n=8$).

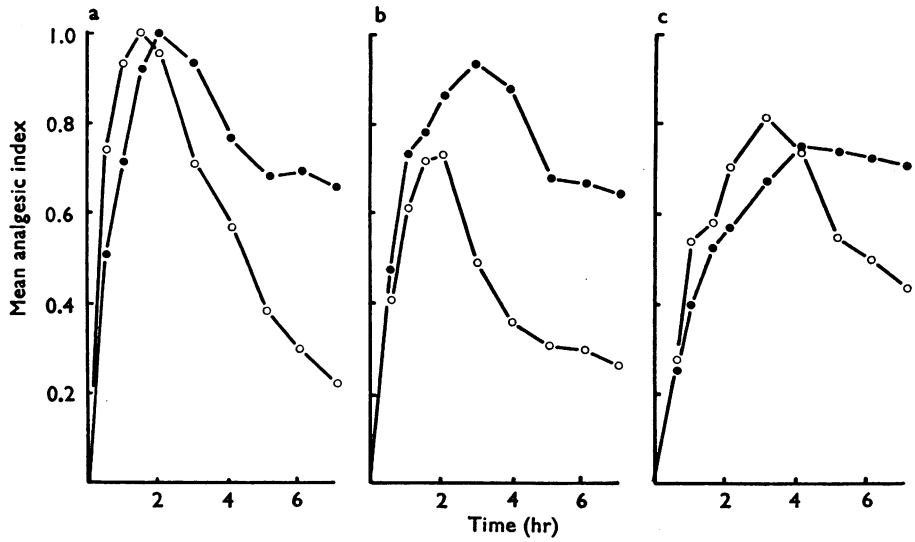

Fig. 5. Effects of actinomycin D on the analgesic responses to infusions of diamorphine, etorphine or pethidine. a: Diamorphine 1 mg/kg/hr; ○, drug alone (n=5); ●, drug plus actinomycin D 10 μg/kg/hr (n=5). b: Etorphine 4 μg/kg/hr; ○, drug alone (n=6); ●, drug plus actinomycin D 10 μg/kg/hr (n=5). c: Pethidine 10 mg/kg/hr; ○, drug alone (n=5); ●, drug plus actinomycin D 10 μg/kg/hr (n=6).

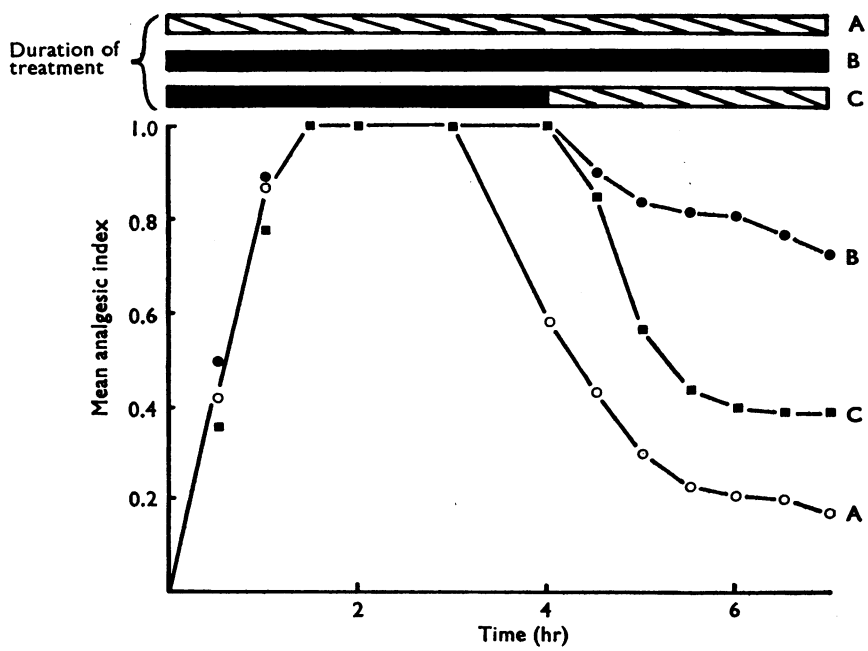

Fig. 6. Duration of the action of actinomycin D. ▨, Periods of treatment with morphine 7.5 mg/kg/hr only; ■, periods of treatment with morphine 7.5 mg/kg/hr plus actinomycin D 10 μg/kg/hr. Four animals in each treatment group (A, B, C). Within 1 hr of the cessation of actinomycin D infusion, its effects are clearly reduced.

than with the other analgesics and so a possible increase in rate of onset might be detected more readily. A group of eight rats were given actinomycin D (10 μg/kg/hr) for 2 hr before pethidine was infused (at 10 mg/kg/hr). Pressure thresholds were measured both before and during the infusions. Comparison of the indices of analgesia obtained from these animals and those obtained from animals receiving pethidine only (Table 1) showed that the actinomycin treatment had not affected the rate of onset of analgesia.

Other experiments were designed to test the possibility that actinomycin D affects the rate of loss of analgesia after the cessation of morphine infusion (that is, in contrast to that seen when the morphine infusion is continued in the phase of declining analgesia). Two groups of animals were infused with morphine (7.5 mg/kg/hr) but only one of these groups received actinomycin D (10 μg/kg/hr) concurrently. After 3 hr when the phase of peak analgesia was reached the morphine infusions were stopped and replaced with saline or, in the case of the animals that had already received the antibiotic, by actino-

TABLE 1

EFFECT OF ACTINOMYCIN D ON THE RATE OF ONSET OF ANALGESIA DURING A PETHIDINE INFUSION

One group of eight animals received an infusion of actinomycin D (10 μg/kg/hr) for 2 hr before the start of, and throughout the course of a pethidine infusion (10 mg/kg/hr). The second group (nine animals) received only pethidine (10 mg/kg/hr)

* Meaningful standard errors cannot be calculated for these observations because the maximum analgesic index value of 1·0 was obtained from one or more animals.

Time from start of infusion (hr)	Mean analgesic index ± S.E.	
	Pethidine + actinomycin D	Pethidine only
0·5	0·22 ± 0·04	0·29 ± 0·03
1	0·41 ± 0·04	0·45 ± 0·04
1·5	0·46 ± 0·03	0·61 ± 0·03
2	0·67*	0·68*
3	0·79*	0·83*

TABLE 2

EFFECT OF ACTINOMYCIN D ON THE RATE OF RECOVERY FORM A 3 HOUR INFUSION OF MORPHINE

Both groups of animals received an infusion of morphine (7·5 mg/kg/hr) for 3 hr. In one group the morphine infusion was followed by infusion of 0·9% sodium chloride solution. The second group received actinomycin D (10 μg/kg/hr) in place of the saline; they had also received actinomycin D at the same rate throughout the course of the morphine infusion.

* Meaningful standard errors cannot be calculated for these observations because the maximum analgesic index value of 1·0 was obtained from one or more animals.

Time (hr) from cessation of morphine infusion	Mean analgesic index ± S.E.	
	Saline treatment (n=5)	Actinomycin D treatment (n=6)
0	0·90*	1·00*
1	0·54 ± 0·05	0·75*
2	0·37 ± 0·06	0·44 ± 0·05
3	0·18 ± 0·03	0·17 ± 0·06
4	0·13 ± 0·03	0·11 ± 0·05

mycin D alone. Table 2 shows the mean analgesic indices in the period of 4 hr after the withdrawal of morphine ; there are no significant differences between the results obtained in the actinomycin treated and control groups.

The effects of actinomycin D on analgesia were studied in animals which had reduced responsiveness to morphine as a result of previous treatment with the analgesic. Eight animals were infused with morphine (7.5 mg/kg/hr) for a period of 11 hr. 6 hr 40 min after the start of the infusion, the mean analgesic index was less than 30% of the peak value ; actinomycin D was then added to the morphine solution infusing four of the animals, while the infusion of morphine alone was continued in the remaining four rats (Fig. 7). While the level of analgesia in rats receiving morphine alone continued to fall, the decline in the analgesic index did not continue in the rats given actinomycin D. At 11 hr the mean index of analgesia from the rats receiving morphine plus actinomycin D was significantly greater ($P<0.05$) than in animals receiving morphine only.

Experiments were also carried out in rats which were infused with morphine (7.5 mg/kg/hr) for 7 hr on each of 4 successive days. There was thus an interval of 17 hr between each period of infusion and the pressure thresholds when determined immediately before the beginning of an infusion were found to have returned to " non-

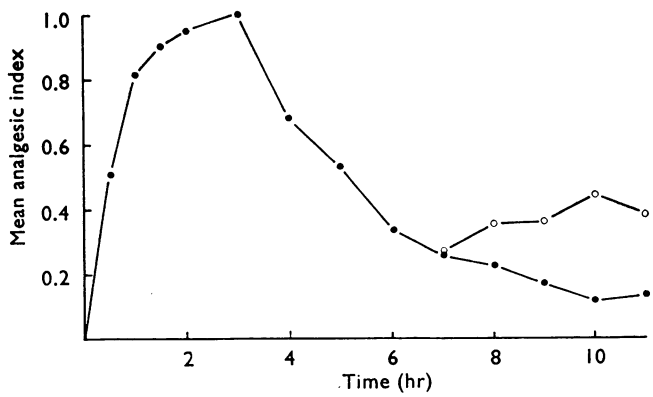

Fig. 7. Effect of actinomycin D in animals showing reduced responsiveness to the analgesic effects of morphine. Morphine 7.5 mg/kg/hr was infused into eight rats. After 6 hr 40 min, four of the animals (○) received in addition actinomycin D (10 μg/kg/hr). The remaining four animals (●) continued to receive morphine only until the end of the experiment.

analgesic " level. (Mean pressure thresholds in cm Hg: day 1, 5.61 ; day 2, 5.45 ; day 3, 5.73 ; day 4, 4.80.) On each infusion day the results obtained from the animals showed the characteristic three phases, increasing analgesia, peak analgesia, followed by declining analgesia. The mean analgesic indices reached during the phase of peak analgesia were however, progressively reduced on each succeeding day of the experiment (Fig. 8). Thus during the fourth infusion the maximum level of analgesia in animals receiving morphine alone was less than 10% of that achieved during the first infusion. On the fourth day, some animals received actinomycin D (10 μg/kg/hr) together with morphine and in these animals the phase of declining analgesia was absent although the peak analgesic response was throughout less than 25% of that shown by animals receiving morphine only on the first day. Following the relatively fast onset of analgesia during the first 2 hr, there was a continued slow increase in the mean analgesic indices obtained from the actinomycin treated animals until the end of the infusion.

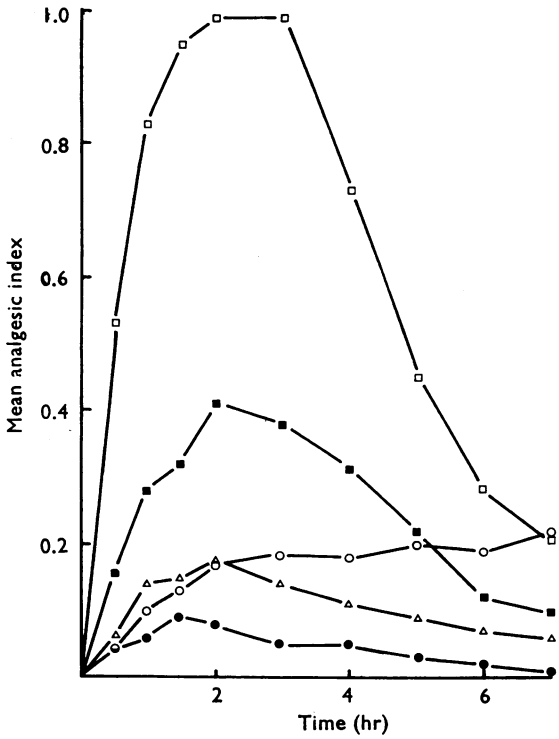

Fig. 8. Mean analgesic indices obtained from animals infused with morphine 7.5 mg/kg/hr) on 4 successive days. □, First day infusion ($n=11$); ■, second day infusion ($n=11$); △, third day infusion ($n=11$). On the fourth day, three animals received morphine only (●) while seven animals received morphine together with actinomycin D 10 μg/kg/hr (○). Each infusion lasted 7 hr.

Effects of actinomycin D

In view of the results described above, it seemed important to determine whether actinomycin D given alone by intravenous infusion at the rate of 10 μg/kg/hr affected the reactions of rats to the pressure stimulus. The results illustrated in Fig. 4 indicate that actinomycin D had neither analgesic nor hyperalgesic activity. The motor co-ordination of fourteen animals which had received actinomycin D infusion for 8 hr, and in fourteen control rats which received saline for the same period, was tested by rotor rod performance. There was no significant difference between the performance of treated and control animals ($P=0.23$).

As an index of the metabolic disturbance that actinomycin D might produce, the urinary total nitrogen excretion was measured in rats during 8 hr infusions of actinomycin D (10 μg/kg/hr) or saline (1 ml./hr). Rats treated with saline excreted 55.3 ± 7.4 μg of nitrogen/100 g body weight/min, while rats treated with actinomycin D excreted 53.4 ± 4.9 μg/100 g/min (means ± standard errors from ten observations in each case). The actinomycin treatment thus had no significant effect on urinary total nitrogen excretion.

DISCUSSION

Development of tolerance during intravenous infusions

During infusions of analgesic drugs at selected rates the peak level of analgesia reached after 2–4 hr was not maintained. The extent of this fall in the level of analgesia during

continued drug infusions was independent of the number of occasions on which the animal was subjected to painful stimuli. Hence it is unlikely to have been the result of increasing sensitivity of the tail as a result of the repeated application of pressure and it is equally unlikely that the rats had become conditioned to respond to an inadvertent conditioning stimulus in the test procedure. Further evidence that the failure to maintain the maximum level of analgesia occurred as a result of the development of tolerance to the drug was provided by the experiments in which morphine was infused to the same group of rats on 2 or more successive days. The level of peak analgesia reached during the second infusion was appreciably lower than during the first experience of the drug and was only a little higher than the level of analgesia recorded at the end of the first infusion. Behavioural changes in the animals during the course of infusions suggested that tolerance was also developing to other actions of the analgesic drugs.

These results thus extend the observations of Martin & Eades (1961) who obtained some indications of the development of tolerance during the course of 8 hr infusions of morphine (3 mg/kg/hr) to conscious dogs. Until measurements have been made of the brain concentration of morphine at various times throughout an infusion of the drug. however, it is uncertain whether the tolerance occurs as a result of a decreased sensitivity of brain mechanisms to the effects of the drug or as a result of an increase in the rate of metabolism and elimination of the drug or alteration in its distribution. The concentration of morphine in the brain after a standard dose is the same in normal and chronically morphinized rats (Jóhannesson & Woods, 1964) so it is unlikely that tolerance is the result of a fall in the brain concentration of the drug.

Tolerance developed very rapidly during the course of an intravenous infusion. With all drugs except pethidine, the level of analgesia had begun to decline within 3–4 hr. With infusions of the highest concentration of pethidine, the phase of increasing analgesia was more prolonged than with the other drugs. It is possible that after infusion at high rates the onset of tolerance was hidden by the increasing dose of drug. The failure of pretreatment with actinomycin D to affect the rate of increase in analgesia during infusions of pethidine at 10 mg/kg/hr (Table 1), however, suggests that, in these conditions, the onset of tolerance was not apparent in the phase of increasing analgesia. The infusion technique seemed to have the advantage over the conventional method of repeatedly injecting single doses to measure the rate of acquisition of tolerance, in that tolerance is developing continuously in, as nearly as possible, constant conditions. During repeated single injections the rate of tolerance development is very much dependent on the dose regimen and the duration of drug action.

Inhibition of tolerance development by actinomycin D

Actinomycin D (10 μg/kg/hr) reduced or prevented the development of tolerance during infusions of analgesic drugs, although when given by itself, it had no effect on the pressure thresholds of rats. When morphine was infused for a relatively short period (3 hr) during which there was no evidence for the development of tolerance, the rate of loss of analgesia when the morphine infusion was discontinued did not depend on whether or not actinomycin D had been infused concurrently.

Presumably the rate of recovery was primarily dependent on the metabolism and elimination of the morphine and much less dependent on the low degree of tolerance

developed during the short infusion. Thus it is probable that actinomycin is not acting by depressing the rate of metabolism and elimination of the analgesic drug. Preliminary experiments have failed to show any significant change in liver pethidine *N*-demethylase activity after infusion of actinomycin D (10 μg/kg/hr) for 8 hr in rats (unpublished observations) and Gelboin & Blackburn (1963) have reported that repeated large doses of actinomycin D (600 μg/kg at 4 hr intervals for 12 hr) had no effect on the activity of a related enzyme of the liver microsomal fraction, benzpyrene hydroxylase. Our failure to detect any change in urinary nitrogen excretion during an 8 hr infusion of actinomycin also suggests that actinomycin D has not caused severe disturbance of normal protein metabolism.

In rats which were tolerant to the analgesic effects of morphine, treatment with actinomycin D did not restore the analgesic response to the level shown in normal animals (see Figs. 7 and 8). Thus "established" tolerance was not reversed by actinomycin although the further development of tolerance was prevented. Actinomycin D seemed to affect a process which occurs only while tolerance is actively developing. The slow progressive rise in the analgesic indices of morphine-tolerant animals receiving an infusion of morphine together with actinomycin D possibly reflects the rate of loss of established tolerance.

A number of other inhibitors of protein synthesis with widely varying chemical structure and differing modes of action also prevent the development of tolerance to morphine during intravenous infusions (unpublished observations). It seems reasonable to conclude that there is an association between the actions of actinomycin D in inhibiting protein synthesis and preventing the development of tolerance to morphine. Actinomycin D inhibits protein synthesis by preventing DNA directed RNA synthesis (Goldberg & Reich, 1964) and there is little doubt that the dose used in the present experiments was sufficient to exert this effect for Fujioka, Koga & Lieberman (1963) have shown that actinomycin D at doses equivalent to 12.5 μg/kg/hr completely prevented the increased uptake of ^{14}C-orotate into liver RNA induced by partial hepatectomy in rats.

We have confirmed the observations of Cohen *et al.* (1965) that actinomycin D can prevent the development of tolerance to morphine, in conditions where there is no widespread metabolic disturbance. Our results therefore support the conclusion that in animals treated with morphine changes occur in the pattern of protein synthesis; these changes are not requisite for analgesic effect, but are causally related to the development of tolerance.

SUMMARY

1. The development of tolerance to the analgesic effects of morphine during intravenous infusions of the drug at selected rates to conscious rats has been demonstrated. Tolerance also developed during infusions of diamorphine, pethidine and etorphine.

2. With morphine, diamorphine and etorphine, some degree of tolerance had already developed within 3–4 hr of the start of infusion. During pethidine infusions the phase of increasing analgesia tended to be more prolonged than with the other drugs.

3. Simultaneous infusion of the protein synthesis inhibitor actinomycin D together with the analgesic drug prevented the development of tolerance. Actinomycin D treatment in the absence of analgesic drug did not affect either the pressure thresholds of the rats or the urinary excretion of nitrogen during the course of an infusion.

4. Actinomycin D did not overcome " established tolerance " in animals that had been previously exposed to morphine, and thus appeared to affect a process which occurred only while tolerance was developing.

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