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Actions of morphine on the segmental reflex of the decerebrate-spinal cat

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Summary

1. The actions of morphine were studied on the segmental reflex of the decerebrate-spinal cat. Morphine decreased arterial blood pressure.

2. Morphine inhibited mono- and polysynaptic reflexes. The influence of morphine on monosynaptic reflexes was more obvious at stimulation of the dorsal root at a frequency of 12·5 Hz than at 0·5 or 2·5 Hz.

3. The total amount of activity recorded from the ventral root after morphine depended on whether or not the evoked reflex was maximal or submaximal.

4. These actions of morphine were antagonized by naloxone.

Introduction

There is disagreement in the literature regarding the actions of morphine on cat spinal cord (Wikler, 1945; Takagi, Matsumura, Yanai & Ogiu, 1955). This communication relates experiments in which we have re-opened the question by studying the actions of morphine on the evoked electrical response of the segmental reflex of the low spinal cat and determining the influence of the magnitude of the evoked response and of stimulus repetition rate.

Methods

Thirty-six cats were used. They were anaesthetized with ether and the trachea, left carotid artery and left jugular vein were cannulated. The head was fixed in a stereotaxic instrument and the skull trephined. The cat was then decerebrated and anaesthesia discontinued (Martin & Eades, 1960). Succinylcholine (20 (μg/kg)/min) was then given by intravenous drip and the cat was respired artificially.

The spinal cord was exposed from L1 to S1 and transected at L2. The last lumbar dorsal and ventral spinal roots were exposed and cut at their point of entry into the dura mater. The cat, fixed by means of the head holder and clamps on the spinal vertebrae, was then raised to prevent contact with the table top. Using skin flaps, the entire area of exposed spinal cord was covered with heavy liquid paraffin of 37° C, equilibrated with 95% O_2 and 5% CO_2. Body temperature, recorded from a rectal thermistor probe, was maintained at 37·5° C. Blood pressure (1 mmHg$\equiv$ 1·33 mbar) was recorded continuously from the left carotid artery by means of a strain gauge transducer and pen oscillograph. No anticoagulant was used.

Stimulation of the dorsal root and recording of the response reflexly evoked in the ventral root were by the techniques described previously (Krivoy, 1957 ; Krivoy & Huggins, 1961). The stimulating and recording electrodes were of bright silver. The

stimulus to the dorsal root was a biphasic pulse of 0·5 ms duration administered through a stimulus isolation unit.

The experiments were conducted in two blocks, each containing 18 cats. In both blocks the stimulus conditions were the same: in 9 cats a stimulus was used which produced a maximal reflex response at one of three frequencies (0·5, 2·5 and 12·5 Hz), and in 9 cats the stimulus was adjusted to produce a submaximal reflex response. Each cat was stimulated at only one frequency. The order of the six stimulus conditions was randomized by means of a latin square. In the first block each cat was injected intravenously with three doses of morphine (0·5, 2·5, and 12·5 mg/kg) and naloxone (1·0 mg/kg). In the second block, three injections of 0·9% NaCl solution (saline) were given, followed by naloxone (1·0 mg/kg).

The strength of stimulus required to evoke submaximal or maximal responses was determined from the response of each preparation to stimulation at 0·2 Hz. This stimulus rate was used to determine that intensity of stimulation above which an increase in stimulus strength did not increase the ventral root response. In one-half of the experiments, the stimulus intensity was then increased by 10% to ensure a maximal response. In the remaining experiments, the stimulus intensity was reduced to such an extent that the response recorded from the ventral root occupied 50–70% of the total area obtained after maximal stimulation. The frequency of stimulation was then increased to 0·5, 2·5 or 12·5 Hz. No further alteration was made in the

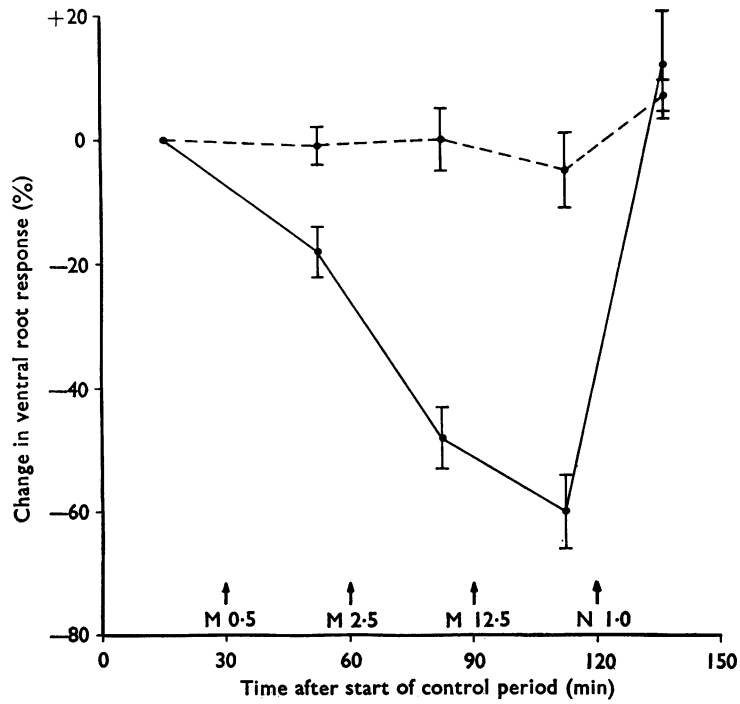

FIG. 1. Effects of morphine (●——●), saline (●- - -●), and naloxone (N) on the total ventral root response, obtained by measuring the area of the potential. Each point represents the mean of the responses of 18 cats. The numbers below the arrows at M indicate the doses of morphine sulphate (mg/kg); at N, naloxone hydrochloride (1 mg/kg). In this and all subsequent figures, the vertical lines indicate the S.E. of the mean. Abscissa, time after the start of the control period; ordinate, change in ventral root response as % of control value.

stimulus parameters, i.e., single stimuli were applied without interruption at intervals of 2, 0·4 or 0·08 seconds.

When the ventral root response had become constant, a 30 min control period was started. Then 0·5 mg/kg of morphine was injected followed after intervals of 30 min by 2·5 and 12·5 mg/kg, and finally by 1 mg/kg of naloxone.

Sequences of ten records of the ventral root responses were photographed at each of the following times: 0, 15 and 30 min during the control period, immediately before and 15 min after each injection, and immediately before and 5, 15 and 30 min after injection of naloxone. To obtain a measure of the total evoked ventral root response the photographic records were enlarged and the tracings of the envelopes of the potentials cut out above the isopotential line and the cut-outs weighed. The data were then normalized separately for each experiment. To do this the values obtained for each sequence of ten traces were averaged and expressed as a percentage of the first control sequence. The differences between the mean of the three control sequences (control mean) and the means of each of the post-drug sequences were obtained. The differences between the control means and the means obtained 15 and 30 min after the various doses of morphine were summed, and the mean obtained. Statistical analysis was then performed on these means. Because the

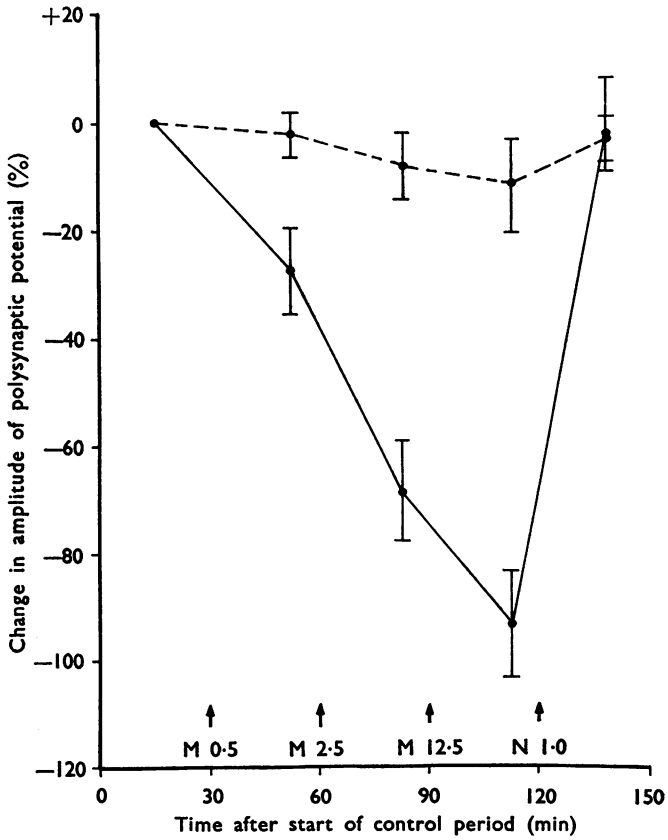

FIG. 2. Effects of morphine (●——●), saline (●– – –●), and naloxone (N) on the peak amplitudes of polysynaptic potentials of the cat. Each point represents the mean response of 18 cats. For further explanation see Fig. 1.

values presented in the figures represent means of observations taken at 15 and 30 min after drug administration, the time of the value given is intermediate between these values, i.e., 22·5 minutes.

An analysis of variance of the data from each block gave the variance between cats which was partitioned into that associated with maximal stimulation, submaximal stimulation, and repetition rate of stimulation; the variance within cats provided the variance due to dose or time. For each analysis the between treatments sums of squares was paritioned into a mean for linear regression which determines the significance of the difference of the slope from zero and a mean square for deviation from linearity.

The effects of naloxone were obtained by comparing the mean of the three post-naloxone values with the means of the pre-morphine or pre-saline values, using the paired *t* test.

In addition, the outlines of each potential were projected on graph paper and the peak amplitude of the monosynaptic spike potential and of one continuously re-appearing polysynaptic spike potential were measured. The values were then treated in the same manner as the weights of the cut-outs.

Systolic and disastolic blood pressures were measured at 5 s intervals before the records of the ventral roots response. These measurements were then treated in the same manner as the weights of the cut-outs.

All drugs were injected intravenously. The doses are expressed as morphine sulphate or naloxone hydrochloride.

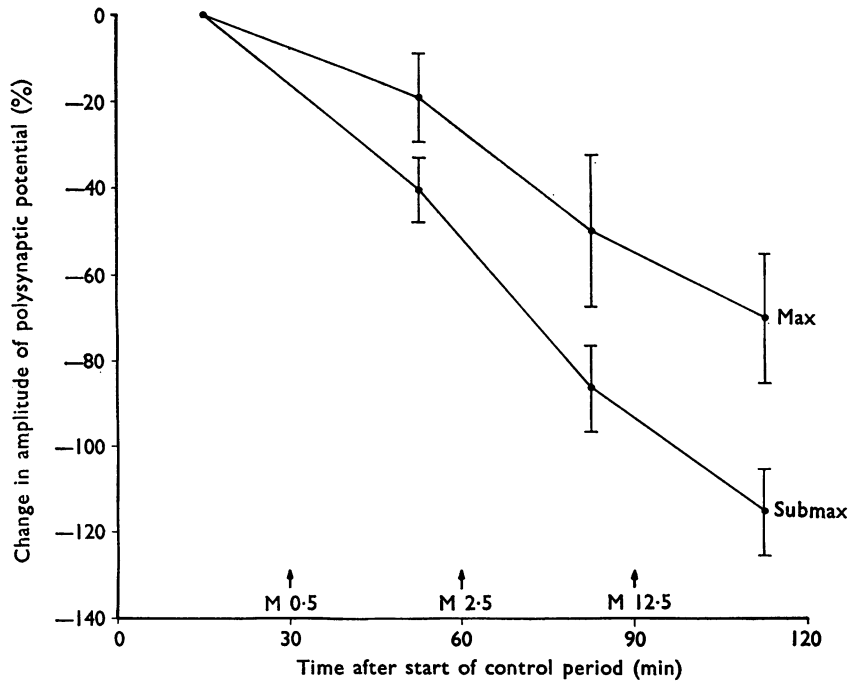

FIG. 3. Effect of morphine on the amplitude of polysynaptic potentials evoked by maximal (Max) and submaximal (Submax) stimulation of the dorsal root. Each point represents the mean response of 9 cats. For further explanation see Fig. 1.

Results

Total evoked responses

The pooled data of the total evoked response to dorsal root stimulation for all stimulus conditions in the saline-treated cats remained constant throughout the experiment (Fig. 1).

In morphine-treated cats, the magnitude of the total evoked response decreased ($P<0{\cdot}01$) progressively with increasing dose (Fig. 1), the curve obtained being non-linear ($P<0{\cdot}01$). There was no statistically significant influence of frequency ($P>0{\cdot}1$), nor was there a difference between maximal and submaximal responses ($0{\cdot}5<P<0{\cdot}1$). After naloxone, the potential recovered to a level not significantly different from that of the control period.

Amplitudes of polysynaptic potentials

Saline injection did not modify the peak amplitude of the polysynaptic potentials (Fig. 2). Naloxone administration failed to alter the amplitude of the response compared with the control value.

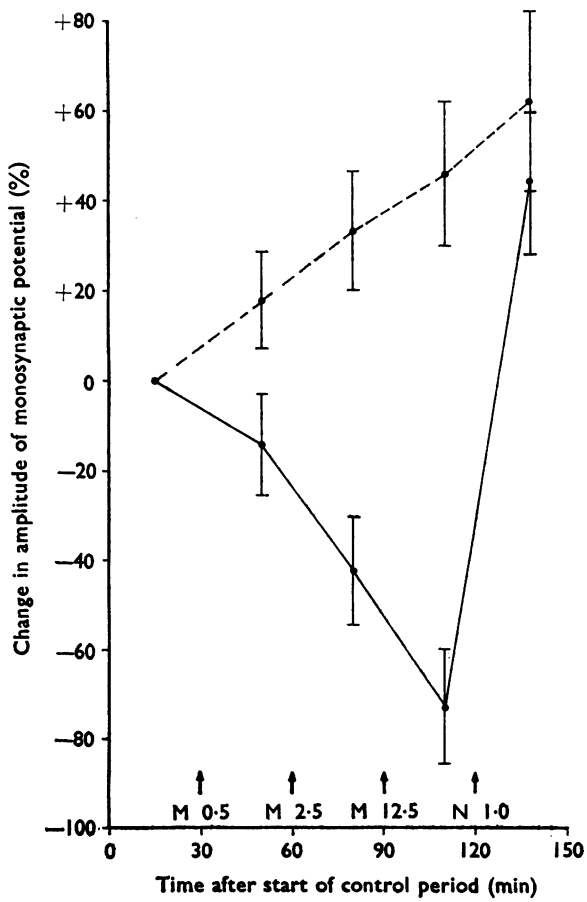

FIG. 4. Effects of morphine (●——●), saline (●– – –●), and naloxone (N) on the amplitudes of the monosynaptic potentials in the cat. Each point represents the mean response of 18 cats. For further explanations see Fig. 1.

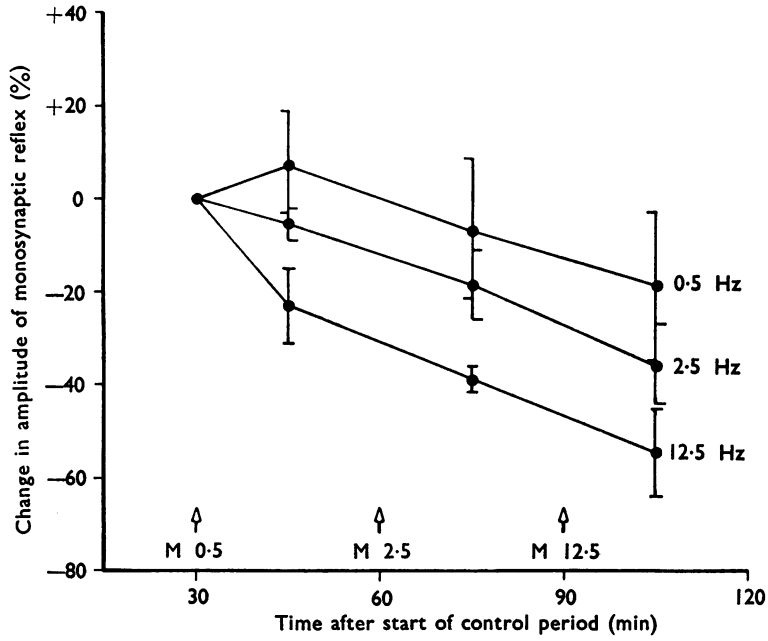

FIG. 5. Effect of morphine on the amplitudes of the monosynaptic potentials evoked by different frequencies of stimulation. Each point represents the mean response of 6 cats. For further explanations see Fig. 1.

Morphine administration diminished ($P<0{\cdot}01$) the amplitude of the polysynaptic response (Fig. 2), the obtained curve being non-linear ($P<0{\cdot}05$). The depression of the amplitude of the submaximal response appeared greater than the depression of the maximal response but the difference was not significant ($0{\cdot}05<P<0{\cdot}10$) (Fig. 3). After naloxone, the amplitude of the polysynaptic potential increased to a level which was not different from that of the pre-morphine control period (Fig. 2).

Amplitudes of monosynaptic potentials

In cats receiving saline, the amplitude of the monosynaptic potential appeared to increase with time (Fig. 4), but this change was not statistically significant ($P>0{\cdot}05$). Similarly, there was no difference between the responses after maximal and submaximal stimulation. On the other hand, the influence of frequency was significant ($P<0{\cdot}05$) ; the increase of the response was greatest at 2·5 Hz and absent at 12·5 Hz.

Morphine caused a significant ($P<0.05$) depression of the amplitudes of the monosynaptic potentials (Fig. 4). When the actions of single doses of morphine were compared for maximal and submaximal stimulation at a given frequency, the submaximal responses were more readily depressed. However, when the data were pooled, this difference was not significant ($0{\cdot}1>P>0{\cdot}05$). The variance due to frequency was significant ($P<0{\cdot}05$). The dose response curves for those frequencies, plotted in Fig. 5, did not deviate significantly from parallelism (Finney, 1964); this may have been due to the relatively small differences and the larger scatter of values. Taken alone, the pooled results for 0·5 Hz did not cause a significant regression ($P>0{\cdot}05$). Naloxone caused a reversal of the morphine-induced depression; the response was significantly

greater than that of the pre-morphine control period ($P<0.05$) but not different from the value observed in the saline-treated cats. There was no difference in the amplitudes of the monosynaptic potentials following naloxone when the morphine-treated cats were compared to the saline-treated cats (Fig. 4).

Blood pressure

In the group of cats receiving saline, neither systolic or diastolic blood pressure changed significantly ($P>0.05$). Naloxone lacked effect on these parameters.

In the group of cats treated with morphine, the systolic blood pressure decreased from 109.0 ± 5.4 to 92.9 ± 7.0 mmHg and the diastolic pressure from 63.2 ± 5.5 to 48.1 ± 5.4 mmHg. Naloxone administration reversed the morphine-induced depression of systolic and diastolic blood pressures, and resulted in a rise of diastolic blood pressure to 75.3 ± 6.3 mmHg, which was above that of the control period ($P<0.05$).

Discussion

From the data presented, it may be seen that morphine depressed monosynaptic and polysynaptic reflexes, as well as the total area of response evoked in a ventral root by stimulation of a dorsal root. Since the observations were made on cats with low spinal sections, the depression could not be the result of morphine activating high spinal or supraspinal mechanisms. Naloxone antagonized the depression, which therefore was likely to be associated with a direct action of morphine, rather than to a mechanism secondary to the deterioration of the preparation with time. However, we cannot ascertain from our results whether these actions of morphine were due to a direct action on spinal synapses, or secondary to the actions of morphine on blood pressure, viscera (Duda, 1964), or stretch receptors (Jurna, 1965).

Wikler (1944, 1945) has reported that, in the spinal cat, monosynaptic reflexes are either not altered or are stimulated, but that polysynaptic reflexes are depressed when the dose of morphine is approximately 5 mg/kg. With larger doses of morphine (15 mg/kg), both monosynaptic and polysynaptic reflexes are depressed for 1·5–2 h. Takagi *et al.* (1955) reported that, in the low spinal cat, morphine has no action either on monosynaptic or polysynaptic reflexes. Since Wikler's preparations were stimulated intermittently, at a rate of less than 0·5 Hz (personal communication), his results did not necessarily differ from those reported in the present paper. Unfortunately, Takagi *et al.* (1955) do not state either the frequency or relative strength of stimulation nor the number of cats studied; this last information may be important because in the present paper, no depression of the polysynaptic potential was found in 3 of 18 cats. Further, Takagi *et al.* observed their preparation for only 5–7 min, and this may explain their failure to find any change ; in Fig. 5 of their paper, the major polysynaptic potential is depressed after morphine.

Our observations on the influence of morphine on blood pressure agree with those of Evans, Nasmyth & Stewart (1952) and also with those of Martin & Eisenman (1962). It is possible that the decreased response obtained after morphine was due to this action on blood pressure. However, this seems unlikely for two reasons. Although Kissel & Domino (1959) reported that the knee-jerk progressively diminished as blood pressure was decreased, this occurred only after the mean arterial blood pressure was lowered by more than 20 mmHg. In our experiments,

the mean fall of systolic pressure was 16 mmHg and that of the diastolic pressure 15 mmHg. Secondly, if the observed changes were due to the reduced blood pressure, they would be in part attributed to tissue hypercapnea and anoxia, leading to a reduction in the ability of the motoneurone to be stimulated antidromically (Brooks & Eccles, 1947; Kolmodin & Skoglund, 1959). From preliminary experiments (Krivoy & Zimmermann, unpublished observations), it would appear that morphine (2·5 mg/kg) does not alter antidromic detonation of the cat spinal motoneurone under conditions identical to those used in our experiments.

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