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Effects of pethidine and nalorphine on the mechanical and electrical activities of mammalian isolated ventricular muscle

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Summary

1. The strength of the isometric mechanical contraction of electrically-driven ventricular muscle has been recorded simultaneously with the resting and action potentials; the effects of pethidine and of nalorphine on these parameters have been studied.

2. When lower concentrations of pethidine (0·22–6·5 μg/ml) were perfused, isometric peak tension was decreased in parallel with the maximum upstroke velocity of the action potential; these actions are considered to result from membrane stabilization. At higher concentrations (11·8–109 μg/ml) pethidine usually produced, in addition, a progressive decrease in the resting and action potentials associated with marked irregularities in, or even abolition of, the mechanical response. It is suggested that these effects of the higher doses might be due to a depression of ATPase activity in the myocardial membrane.

3. Compared with pethidine, nalorphine had similar, but weaker, actions.

Introduction

The following circulatory actions of pethidine and of nalorphine have been reported previously (Grundy, 1971). In the anaesthetized cat or rabbit, after intravenous doses of 2–10 mg/kg pethidine, the blood pressure responses typically consist of transient pressor or depressor changes preceding a more prolonged hypotensive phase, which is the result of direct musculotropic peripheral vasodilation and cardiac depression. In respect of this latter effect, on the Langendorff preparation of the guinea-pig heart a single administration of pethidine (10 μg–2 mg) predominantly produces a dose-dependent decrease in the amplitude of the mechanical contraction accompanied, at the higher doses, by bradycardia and cardiac irregularities including arrest of the heart. In these preparations nalorphine, when given alone, acts like pethidine but is a much weaker agonist. Although nalorphine, in up to five times the pethidine dosage, does not usually prevent the cardiovascular actions of pethidine administered subsequently, it can mollify these effects if given when they are already present.

In this paper, the actions of pethidine and of nalorphine have been studied on the simultaneously recorded mechanical and electrical activities of mammalian isolated ventricular muscle. A preliminary account of the changes elicited by pethidine in some relevant parameters has already been reported (Grundy & Tritthart, 1971).

Methods

The following preparations (numbers in parentheses) were used: papillary muscle of the cat (9), guinea-pig (13), and rhesus monkey (3); trabecula carneae of the cat (4). Guinea-pigs were stunned by a blow on the head or, like cats and monkeys, deeply anaesthetized with ether. Immediately, the chest was opened and the heart transferred to a dissecting dish containing well-oxygenated Tyrode solution of the following composition (mM): NaCl 136·9; KCl 2·68; $NaHCO_3$ 11·9; $CaCl_2$ 1·8 (guinea-pig) or 2·5 (cat and monkey); NaH_2PO_4 0·42; D-glucose 5·6. The right chambers of the heart were opened and the relevant ventricular muscle, with the corresponding chordae tendinae and part of the tricuspid valve in the case of a papillary muscle, excised. The muscle (0·3–0·7 mm thick) was fixed towards one end of the recording bath, which was perfused at a constant rate (5–10 ml/min) with Tyrode solution at $35 \pm 0{\cdot}2°$ C, equilibrated with oxygen containing 5% CO_2. The bath capacity was 1·3 ml. The unattached end of the muscle (the valvular part of a papillary muscle) was then connected by a bent needle to the core of a linear-displacement transducer (GLC, Type SS—201, Collins Corporation). A weight of 1 g displaced the transducer pin by 0·1 mm. The muscular contraction was practically isometric, the maximum shortening being $<1\%$ of the muscle length. The muscle was stimulated, at the end opposite to the mechanical recording attachment, through two silver wire electrodes 1 mm apart in the floor of the bath, with rectangular pulses generated from a stimulator which also triggered the oscilloscopes (Tektronix, 502A for display and 564B, storage, for photography). The signal produced by the transducer was transmitted through a calibration unit to the oscilloscopes and, via an analogue differentiator, to a pen recorder (Linearcorder WTR 281, Watanabe). Stimulation at a rate of 2 Hz was by pulses of 0·4–5 ms duration and of strengths 1–2 times threshold. With each muscle, when suitable stimulation parameters had been decided, the resting length was increased until further stretching produced no significant increment in the maximum height of the mechanical response. Action potentials were recorded by means of freely-suspended glass microelectrodes of tip resistance 5–20 MΩ; the preamplifier was accompanied by circuits which allowed capacity compensation of the whole of the microelectrode assembly and analogue differentiation of the upstroke velocity of the action potential.

The perfusion arrangement was such that changeover from one solution to another could be effected without any significant alteration either in the flow rate or in the temperature of the perfusing fluid. After a control period of 30 min, provided that the amplitude of the mechanical contraction and the measured electrical parameters remained constant, a drug solution was perfused usually for a period of 10 min, at the end of which the pure Tyrode solution was perfused for at least 20 min to wash out the drug. Composite photographs, each showing the mechanical contraction and the complete action potential and, at a faster speed, the rising phase of the action potential or its differentiation or both were taken from the storage oscilloscope at least at the following times: (1) during the initial control period, (2) at the points of the greatest alterations in the mechanical and in the electrical activities under the influence of the drug, and (3) when maximum recoveries from the drug action had been achieved. Drugs were never administered more often than every 30 min and, with high doses, even less frequently. In the experiments designed to show antagonism between pethidine and nalor-

phine, the latter drug was perfused alone within 5 min before, or concurrently with, or alone 4–10 min after, the beginning of a pethidine administration.

The following drugs were used: lignocaine hydrochloride monohydrate (Pharma-Stern; M.W.289); nalorphine hydrobromide (Burroughs Wellcome; M.W.392); pethidine hydrochloride (Roche; M.W.284). Each day they were freshly dissolved in the appropriate Tyrode solution. The final concentrations are expressed as μg/ml or μM of the free base.

Results

There were no obvious differences in the responses obtained with either the papillary muscle of the cat, guinea-pig or rhesus monkey or the trabecula carneae of the cat; accordingly, the results from all these preparations have been combined and are referred to, subsequently, under the generic title 'ventricular muscle'.

Effects on the mechanical activities of ventricular muscle

The isometric peak tension of ventricular muscle was usually decreased (negative inotropic effect) by pethidine to a degree dependent upon the drug concentration from a threshold of 0·22 μg/ml (0·88 μM) up to 109 μg/ml (440 μM) (Fig. 1). Often, however, especially when the muscle had recently been set up, a positive inotropic effect complicated the response and this could occur either as the only effect or in combination with a negative inotropic action. The degree of this stimulation was most commonly up to 15% of the initial control contraction but it was not consistent at any given pethidine concentration. Therefore, in the whole series of experiments it was impossible to assess the absolute negative inotropic effects produced by different doses of pethidine. Nevertheless, the values for the depressant effects lie along a typical dose-response curve. The phases of

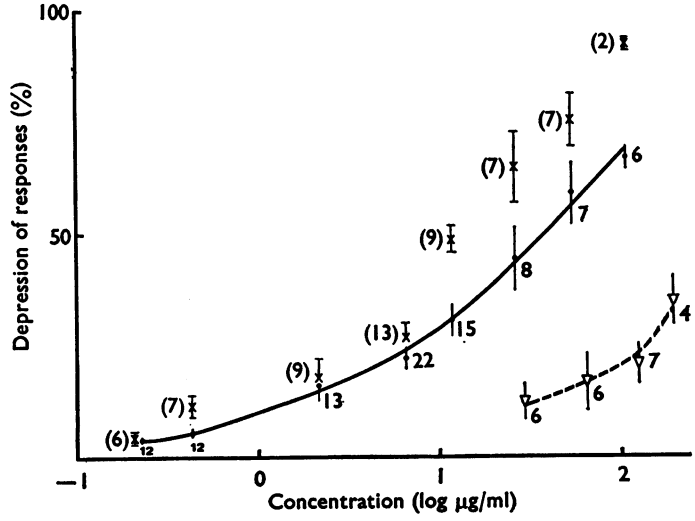

FIG. 1. Concentration-response curves for pethidine (●——● or ×) and for nalorphine (▽ - - - - - - ▽) in respect of their maximum depressant effects on either the isometric peak tension or the maximum upstroke velocity produced during 10 min perfusions of a ventricular muscle preparation. Abscissae, concentrations of drugs (log μg/ml). Ordinates, depression (%) of peak tension (●, ▽) or of maximum velocity of upstroke of action potential (×). Each point represents the mean of the number of trials shown; vertical lines indicate ±S.E. of means.

positive inotropic action were often, but not invariably, accompanied by effects indicative of catecholamine release, that is, decreases in the diastolic lengths of the muscles and increased rates of the mechanical relaxations in relation to those of the corresponding contractions.

At the lower concentrations (0·22–6·5 μg/ml) of pethidine, recovery from the negative inotropic effect and a return to the initial peak tension occurred either during the 10 min of drug perfusion or rapidly within the washout period. With higher pethidine concentrations (11·8–109 μg/ml), subsequent perfusion with drug-free Tyrode solution either restored the mechanical activity more slowly to its original peak tension or never achieved this effect. Further, as the concentration of the drug was raised, there was an increasing tendency for the production of cardiac irregularities whilst pethidine was present or during the washout period. Minor changes in the heart muscle action were occasionally seen with lower concentrations of pethidine and most commonly manifested themselves as missed beats followed by extrasystoles (Fig. 2, A, B and E); with high concentrations cardiac standstill (Fig. 2, C and D), could occur or spontaneous myocardial

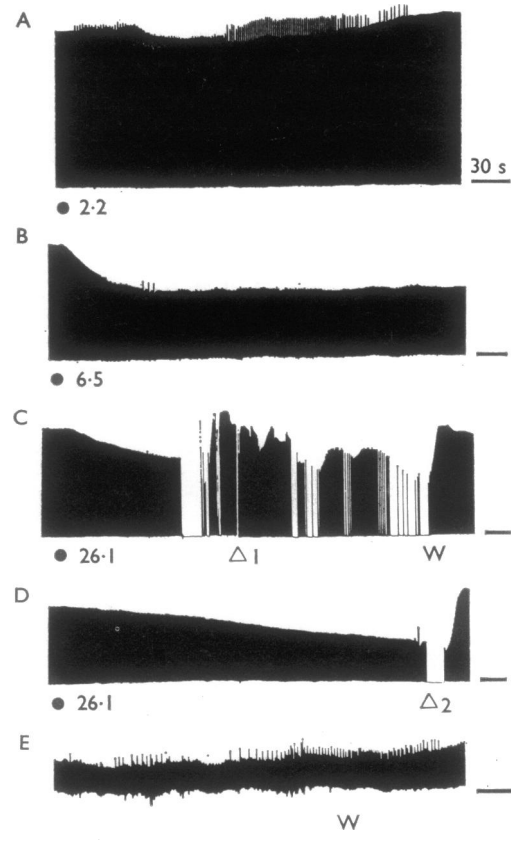

FIG. 2. Irregularities in ventricular muscle contractions produced by pethidine. ●, Addition of pethidine to perfusion fluid, numbers indicate concentrations (μg/ml); W, washout. In (C), at $\triangle_1$, the strength of stimulation was increased from 0·5 to 1 V with an unchanged duration of 2·6 ms; in (D) at $\triangle_2$, the duration of each stimulus was prolonged from 0·4 to 4 ms for a period of 20 s at an unchanged strength of 1·5 V. In (E) the tracing began 3 min 17·5 s before the end of a 10 min period of perfusion with 54·5 μg/ml pethidine. Horizontal lines, 30 s.

activity develop (see below, pacemaker potentials). When contractions of the muscle stopped, they could usually be restarted by increasing the strength (Fig. 2, C) or the duration (Fig. 2, D) of the stimulus.

Nalorphine (30–195 μg/ml; 96·5–627 μM) acted in a manner similar to pethidine although its effects were weaker (Fig. 1). When given in a concentration 1–2 times that of pethidine, 3–5 min before a perfusion with pethidine, nalorphine did not prevent the depressant action of pethidine; when administered simultaneously, the effects of the two drugs were additive but, when perfused at the time of the maximum effect of pethidine, a relatively low nalorphine concentration improved the peak tension in a way similar to that produced by the perfusion of drug-free Tyrode solution.

Effects on the electrical activities of ventricular muscle

Electrical recordings were only acceptable when the resting and action potentials varied by less than 3 mV for two successive minutes before exposure to the drug and returned to the same values and range following washout, which was indicative of a persistent micropuncture of a single cell. When this criterion was satisfied it was found that in the presence of lower concentrations of pethidine (0·22–6·5 μg/ml), the most marked and consistent electrical effect was a dose-dependent diminution in the upstroke velocity, usually with a corresponding impairment of the rate of conduction and of excitability but without any significant decrease in either the resting potential or the height of the action potential (Fig. 3). The fall in upstroke velocity could be detected even when the mechanical tracing showed a positive inotropic effect. The development with time of the depressant actions of the drug on the isometric peak tension and upstroke velocity in the absence of any notable variation in the resting potential is shown in Fig. 4, A; these effects are similar to those produced by a perfusion with lignocaine (43·2 μg/ml or 185 μM; Fig 4, B).

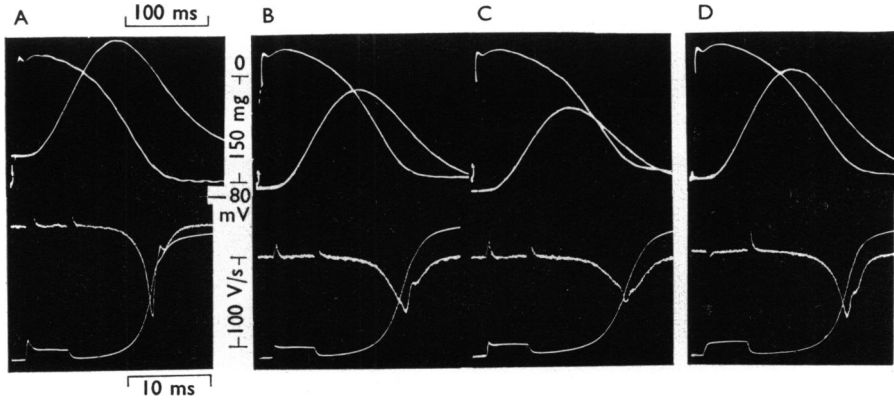

FIG. 3. Effects produced on the mechanical and electrical parameters of a cat papillary muscle by a relatively low concentration of pethidine (6·5 μg/ml). The upper tracings of each vertical pair depict the action potentials and the isometric contractions and the lower tracings, at a faster speed, the rising phases of the action potentials (beginning below) and the differentiations of this electrical parameter. (A) Control; (B) 1 min and (C) 2 min after addition of pethidine to the perfusing fluid; (D) 3 min after changing to drug-free Tyrode solution. The depression of the isometric peak tension reached its maximum 2 min after addition of pethidine; recovery of all three parameters was complete 15 min after changing to drug-free Tyrode solution.

With higher concentrations of pethidine (11·8–109 μg/ml), the combination of effects just described sometimes appeared as the whole response. In the majority of experiments, however, it occurred only in the initial phase (Fig. 5, B) and the

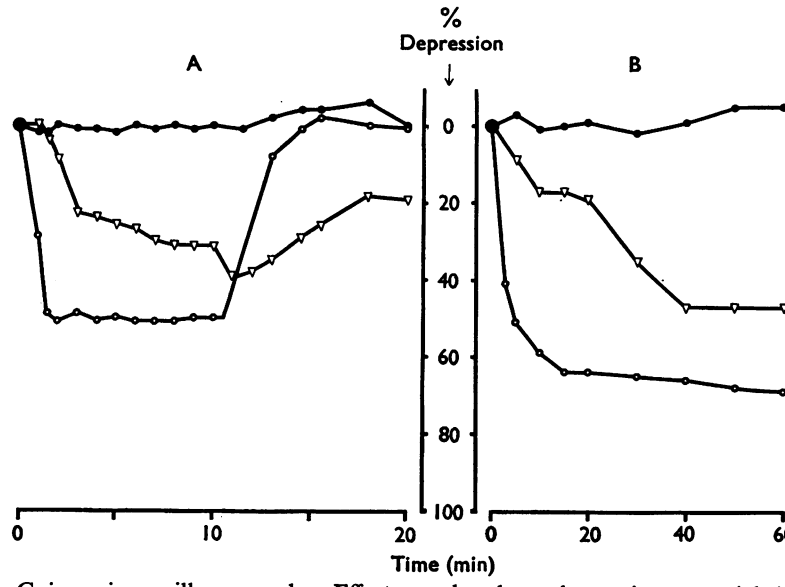

FIG. 4. Guinea-pig papillary muscle. Effects produced on the resting potential (●——●), the maximum upstroke velocity (▽——▽) and the isometric peak tension (○——○) by the addition to the perfusion fluid of (A) pethidine, 26·1 μg/ml (106 μM) for 10 min, followed by washout with Tyrode solution, and (B) lignocaine, 43·2 μg/ml (185 μM) for 600 minutes. Abscissae, time (min). Ordinates, depression (%) of the three parameters.

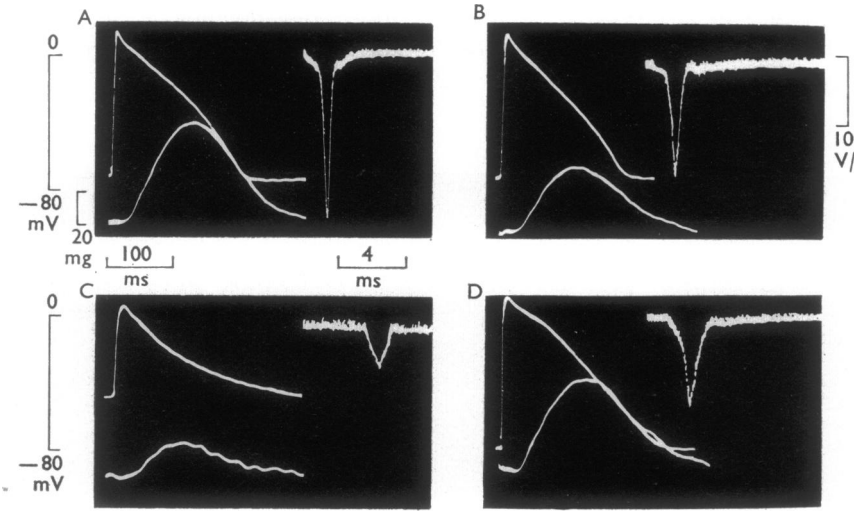

FIG. 5. Effects produced by pethidine on the contractions (beginning lower left on each tracing), the action potentials (upper left) and the differentiated upstroke velocities (right of each tracing) of a guinea-pig papillary muscle, stimulated every 500 ms with a pulse of 0·47 ms duration and twice the threshold strength. (A) Control; (B) after 1 min perfusion and (C) after 10 min perfusion with Tyrode solution containing pethidine (54·5 μg/ml) which was replaced 30 s later by drug-free Tyrode solution; (D) 15 min 30 s later. The differential upstroke velocity tracings show the time of conduction of the impulse from the point of stimulation to the recording site as the time interval between the beginning of the tracing and that of the upstroke of the action potential.

characteristic and predominant effects were decreases in the resting potential and in the height of the action potential together with a marked fall in upstroke velocity (Fig. 5, C). At such high concentrations of pethidine, the electrical changes almost invariably took longer to develop than the depression of the contraction (Fig. 4, A) but, once present, were also more persistent than the latter, except when gross mechanical abnormalities had supervened. For example, during the washout period from the drug, the upstroke velocity could still be decreasing while the mechanical response was recovering (Fig. 4, A) or, when the latter had regained its original value, the former was often still markedly reduced (Fig. 5, D). Note further the prolonged slowing of conduction produced by high pethidine concentrations (Fig. 5), which on three occasions also induced pacemaker potentials heralding the onset of spontaneous mechanical activity. Although there was often an increased duration of the action potential during the phase of positive inotropism, which tended to predominate during the perfusion of smaller doses, no consistent effect was observed over the whole range of pethidine concentrations.

The results described so far were obtained with a stimulation frequency of 2 Hz. In two further experiments, the frequency-dependence of the upstroke velocity was tested during the prolonged perfusion of pethidine in concentrations of 6·5 and 26·1 μg/ml. It was found that, in contrast to the effect produced in drug-free Tyrode solution when the maximum upstroke velocity was only slightly affected by increasing the rate of stimulation from 2 to 8 Hz (Fig. 6, A), in the presence of pethidine the upstroke velocities, already depressed by the drug, showed a further marked depression as the frequency was increased (Fig. 6, B and C).

In a single series of experiments, the electrical modifications induced by relatively high concentrations of nalorphine (30–195 μg/ml) were found to be similar to those of lower concentrations (0·22–6·5 μg/ml) of pethidine. That is, these

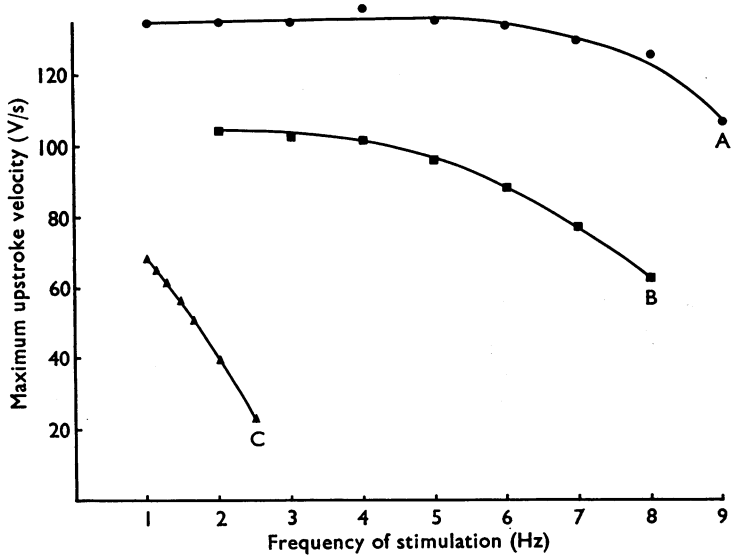

FIG. 6. Frequency-dependence of the maximum upstroke velocity of the action potential in papillary muscles (A and C, cat; B, guinea-pig). (A) Control in drug-free Tyrode solution; (B) after perfusion with pethidine (6·5 μg/ml) for 16 min; (C) after perfusion with pethidine (26·1 μg/ml) for 1 hour.

nalorphine concentrations produced decreases in the upstroke velocity without significant changes in either the resting potential or in the height of the action potential. Perfusion with nalorphine 125 μg/ml for 19 min elicited a frequency-dependence of the maximum upstroke velocity similar to that found for pethidine.

Correlation of the effects of pethidine on the mechanical and electrical activities of ventricular muscle

Figure 1 shows that, with lower concentrations of pethidine (0·22–6·5 μg/ml), the maximum velocity of the upstroke of the action potential and the peak tension were similarly depressed. But, at higher concentrations (11·8–109 μg/ml), although the mechanical activity and upstroke velocity might initially be decreased in parallel (Fig. 5, B), within 10 min of the beginning of the perfusion an additional factor appeared which diminished the upstroke velocity preferentially (Fig. 5, C), so that the maximum depressions of this latter parameter became greater than those of the peak tensions (Fig. 1). In Table 1 the major effects characteristic of lower and of higher concentrations of pethidine are correlated.

Discussion

Whilst this paper is primarily concerned with the depressant effects of pethidine on the myocardium, mention must first be made of the initial phase of positive inotropism which has often been encountered. On the basis of earlier experiments (Grundy, 1971) with pethidine on more intact preparations, especially the Langendorff preparation of the guinea-pig heart, this initial stimulation of cardiac contraction produced by pethidine and by nalorphine might possibly result from the release of endogenous catecholamines or histamine or of both.

Although our studies of the cardiodepressant actions of pethidine were often complicated by the initial positive inotropic effect, sufficient significant data have been obtained to allow the following conclusions to be drawn. The effects produced by lower concentrations (0·22–6·5 μg/ml) of pethidine, namely, a decrease in the contraction of the ventricular muscle associated with a concomitant, parallel

TABLE 1. *Correlation of the effects typically produced on the mechanical and electrical activities of ventricular muscle by pethidine*

Parameter measured	Effects of pethidine		
	Lower concentrations (0·22–6·5 μg/ml.)	Higher concentrations (11·8–109 μg/ml.)	
		Initially	Later
Mechanical activity			
Isometric peak tension	–	- -	- -
Irregularities of beat	o/+	o/+	o/++
Spontaneous activity	o	o	o/+
Electrical activity			
Maximum upstroke velocity	–	- -	- - -
Conduction of the impulse	–	- -	- - -
Excitability	–	- -	- - -
Resting potential	o/+	o/+	- - -
Height of action potential	o	o	- - -
Duration of action potential	±	±	±
Induction of pacemaker potentials	o	o	o/+

Explanation of symbols: o, negligible; ±, variable; +, mild (cardiac irregularities) or present (spontaneous activity, pacemaker potentials) or small increase (resting potential); ++, severe (cardiac irregularities). Depression: –, significant; - -, pronounced; - - -, extremely marked.

diminution in the upstroke velocity of the action potential but with no other significant basic electrical changes, are explicable if we assume that, at these concentrations, pethidine acts as a membrane stabilizer. This conclusion is reinforced by the observation that the local anaesthetic, lignocaine, elicits a similar combination of effects.

If this view is correct the depression of sodium ion movement across the cardiac cell membrane would manifest itself primarily as a fall in the maximum upstroke velocity of the action potential whilst the concomitant hindrance of calcium ion entry would produce an accompanying diminution in the amplitude of the myocardial contraction, that is, a negative inotropic effect. The former action could, however, occur alone when the negative inotropic effect was offset by a positive inotropic action due to catecholamine or histamine release. Further, the rate of conduction through the cardiac tissue would also fall because of the membrane-stabilizing action of pethidine. On the other hand, the lessening of the passages of both potassium and sodium ions through the heart cell membrane would tend to oppose each other in respect of the resting potential which, accordingly, would remain essentially unchanged. Higher concentrations of nalorphine are required to produce a similar membrane stabilization.

Tritthart (1971) found that with some anti-dysrhythmic drugs there was a marked fall in the upstroke velocity of the action potential as the stimulation frequency to ventricular muscle was progressively raised from 2 Hz. The similar frequency-dependence of the maximum upstroke velocity which we have observed after prolonged perfusions with both lower (6·5 μg/ml) and higher (26·1 μg/ml) concentrations of pethidine and with nalorphine (125 μg/ml) suggests that the membrane-stabilizing effects of these drugs occur over a wide range of doses and are present alongside the effects described in the next paragraph.

Higher concentrations of pethidine (11·8–109 μg/ml), at a stimulation frequency of 2 Hz, produced additional actions which, by comparison with similar effects of high concentrations of ouabain, could possibly be due to a depression of the myocardial $Na^+K^+Mg^{++}$-dependent ATPase. This would cause a decrease in the resting potential, and successively engender the following series of phenomena which we observed. First, it would further diminish the maximum upstroke velocity—out of proportion to the decrease in the mechanical contraction—and decrease the action potential height. Secondly, as the depolarization approaches the critical level for the generation of an action potential, irregular beats or spontaneous automaticity (see Vassalle, Karis & Hoffman, 1962) could occur. However, finally, and more commonly at this stage, there was a phase of markedly diminished excitability or conduction which could progress to cardiac arrest.

In summary, it is suggested that the changes in the mechanical and electrical activities of ventricular muscle which we have observed on the application of increasing concentrations of pethidine are explicable if this drug first of all causes partial membrane stabilization and then, in the higher doses which we used, also causes a progressive depression of the cardiac membrane ATPase activity. Nalorphine, a weaker agonist in this connexion than pethidine, is thought never to have been present in a sufficiently high concentration to have achieved the latter effect.

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