between their exposed tips measured 0.5 mm. The substantia nigra was stimulated with a 0.1–0.15 msec pulse of 4.5–5.0 V. The conditioning stimulus to the substantia nigra preceded the test stimulus by an interval varying between 1 and 1000 msec. After a waiting period of 75 sec or longer, the next stimulus combination was given. At the end of the experiment the brain was removed and prepared for frozen sections. All stimulating electrode placements were verified histologically.

Results and discussion. Stimulation of the substantia nigra resulted in an increase in amplitude of primary components of visual, auditory, and somatosensory evoked responses in cortical primary receiving areas bilaterally. This is illustrated in Figure 1. The upper row shows the visual evoked response (C), which was enhanced by nigral stimulation (T). The middle, and lower rows represent auditory, and somatosensory evoked responses, respectively. Both were enhanced by conditioning stimulation of the *substantia nigra* (T). Nigral stimulation alone produced no response in each recording site.

The time course of effects of nigral stimulation on the visual evoked response in the ipsilateral lateral gyrus is shown in Figure 2. The left graph presents the positive component of the response and the right graph the negative one. ○ ● ▼ ■ represent values from different animals, and each electrode placement is illustrated in the lower diagram. Polyphasic curves could be seen, and a marked increase in amplitude of the response occurred at conditioning-test intervals of 9–19, 30–40, 60–75, and 410–590 msec. Figure 3 shows the time course of effects of nigral stimulation on the visual evoked response in the contralateral lateral gyrus. The effective conditioning-test interval ranges were the same.

Modification of sensory impulses both by the reticular formation and by the nonspecific thalamic nuclei has been well established[6–9]. In the last decade effects of caudate stimulation on sensory activities have been studied[10–12]. In our previous studies[2–4] enhancement of photically evoked potentials by lenticular, caudate, and rubral stimulation was observed, and in this investigation that of visual, auditory, and somatosensory evoked responses by nigral stimulation was found. The anatomical findings[13–15] that the *substantia nigra* receives afferent fibers from the *striatum, globus pallidus*, etc., and sends efferents to the *striatum, globus pallidus*, reticular formation, etc. seem to support the results obtained in the present study. The first effective conditioning-test interval range of nigral stimulation is similar to that of rubral stimulation[4], and longer than that of lenticular and caudate stimulation[2,3]. The second, third, and fourth ones are similar to those of lenticular, caudate, and rubral stimulation[2–4]. This suggests an intimate functional relation between these structures.

Since it has been asserted that the destruction of the *substantia nigra* is responsible for parkinsonism, its role in motor function has been investigated[1]. On the other hand, TATETSU[16] reported psychological symptoms, such as personality changes, disturbance of the self, and delusions, that were associated with damage of the *substantia nigra*. From this and our findings, it can be said that the *substantia nigra* functions as 'nonspecific' in the brain stem.

Zusammenfassung. Nachweis einer «unspezifischen» fördernden Wirkung von Stimulation der Substantia nigra auf «evoked potential» in den primären sensorischen Zentren des Grosshirns.

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Effects of Octanoate on the Electrical Activity of Purkinje Fibres

Much discussion has recently centred around a possible arrhythmogenic effect of increased circulating free fatty acid (FFA) levels in acute myocardial infarction. On one hand, an association between high serum-FFA concentrations and incidence of serious arrhythmias and death has been found in patients with coronary occlusion[1], and even a causative role for increased FFA-levels has been suggested by results obtained in dogs with experimental infarction[2,3]. other observations, however, argue against such a direct arrhythmogenic effect of FFAs both in clinical situations[4,5] and under experimental conditions[6]. Increased ectopic pace-maker activity, orginating mainly from terminal Purkinje fibres, is known to play an important part in the development of severe ventricular arrhythmias following acute coronary occlusion[7]. The present investigation was undertaken, therefore, to study the effect of a FFA on the electrical activity of Purkinje fibres. To perform such experiments seemed worth-while, all the more because no information is as yet available on the electrophysiological effects of FFAs in single cardiac fibres. The preliminary results to be

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presented in this paper demonstrate that one of the medium chain FFAs, octanoate, is capable of profoundly altering the action potential characteristics of calf Purkinje fibres. Some of the changes observed might be relevant to a potential arrhythmogenic effect.

Materials and methods. Purkinje fibre-ventricular muscle preparations were dissected from the right ventricle of calf hearts obtained from the slaughter house. The preparations were pinned under slight tension to a soft plastic block in a tissue bath containing modified Locke's solution at 32 °C. Transmembrane potentials were recorded from small terminal radicles of Purkinje fibres by using a standard microelectrode technique, as described earlier[8,9]. The preparations were electrically driven at a rate of 60/min. In some experiments the spontaneous activity of right ventricular strips was also followed continuously by recording surface potentials through bipolar platinum electrodes on a pen-recorder (Helcoscriptor). The nutrient solution contained (m*M*): NaCl 125; KCl 2,8; $CaCl_2$ 2.16; $NaHCO_3$ 25; glucose 11, and was gassed with 95% O_2 and 5% CO_2 (pH 7.4). The relatively low potassium concentration was used to promote the development of spontaneous activity. Octanoate was prepared with NaOH from octanoic acid (Fluka). Taking into account the results of earlier experiments[10,11] and clinical observations[1], octanoate was used in a high concentration (2.4 m*M*). Statistical significance was calculated by Student's *t*-test. Mean values ± S.E. have been given.

Results and discussion. The most striking effect of octanoate (2.4 m*M*) on the transmembrane potentials of electrically driven Purkinje fibres was a very marked shortening of the action potential duration, chiefly due to a decrease in the plateau length. At the same time, the early rapid phase of repolarization became more pronounced. Exposure of the fibres to octanoate for 60 min did not result in any change in the resting membrane potential, whereas a slight decrease in the amplitude of the action potential was obvious. The maximum rate of depolarization was reduced nearly by half, and there was a decrease in conduction velocity. The changes were reversible; after 60 min washing with control solution, a significant recovery was apparent. A typical experiment in which it was possible to observe these time-dependent alterations in the same cell is shown in Figure 1. Statistical analysis of some characteristics of the action potentials recorded under control conditions (n = 20) and after 60 min exposure to octanoate (n = 16) has revealed that the 50, 70 and 90% repolarization times decreased from 317 ± 0,6, 341 ± 0,8 and 361,1 ± 1 msec to 187,6 ± 2,1, 223,4 ± 1,8 and 241,6 ± 4,2 msec. respectively. The maximum rate of depolarization was reduced from 520,7 ± 1,8 to 275 ± 1,5 V/sec, and the action potential amplitude from 116,8 ± 0,4 to 107,6 ± 0,5 mV. All these changes were statistically significant ($p < 0{,}001$). The resting membrane potential amounted to 90,4 ± 0,2 mV under control conditions and it was essentially unchanged in the presence of octanoate (98,8 ± 0,3 mV).

It is not yet known exactly what changes in the electrical activity of a single cardiac fibre are causally related to the onset of most arrhythmias encountered in the clinic. However, the major effects of octanoate (i.e. the shortening of the action potential duration and the decrease in rate of depolarization and conductivity of Purkinje fibres) are known to be present in numerous conditions

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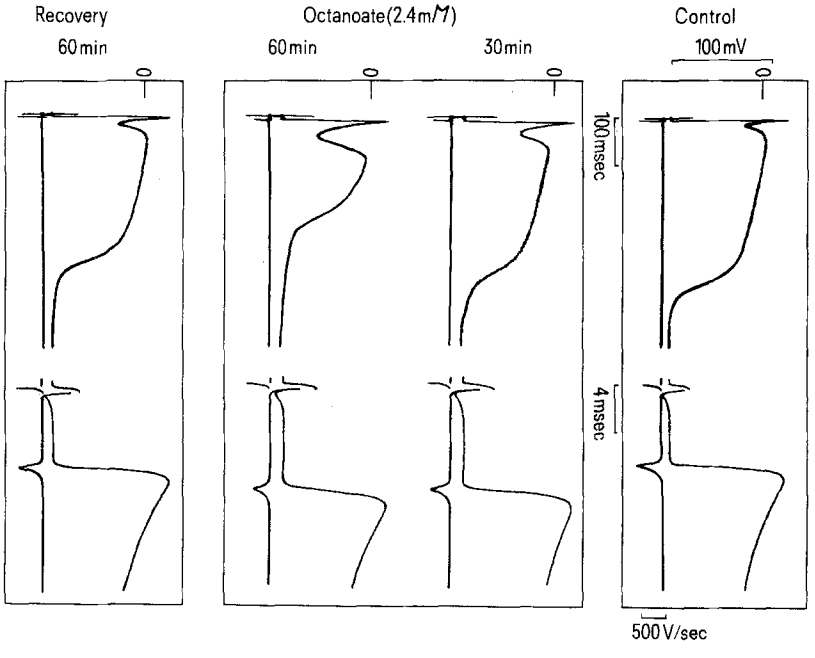

Fig. 1. Effects of octanoate on the intracellularly recorded action potential of a calf Purkinje fibre. In each panel, upper traces: stimulus artefact from driving electrode on Purkinje fibre and the same intracellular action potential at slow (left) and fast sweep speeds (right); bottom traces: stimulus artefact from driving electrode on Purkinje fibre and the differential of intracellular record, the amplitude of this spike being proportional to the maximum rate of depolarization (dV_{max}/dt). The interval between the stimulus artefact and the differentiated record gives an estimate of conduction time. O: zero potential. The calibration indicated at control traces is the same for each panel. Driving frequency: 60/min.

e.g. anoxia[12], halothane anaesthesia[13], etc) which can precipitate the development of dysrhythmias. A decrease in conduction, even in a short segment of Purkinje fibres, can increase the probability of arrhythmias in many ways[14], and the shortening of the action potential duration, especially together with the slowing of conduction, might be expected to favour re-entry activity[15]. Another mechanism by which octanoate could promote the tendency towards arrhythmias is if it did not shorten the action potential duration of adjacent cardiac fibres or groups of fibres to the same extent. This might result in an increase in the normal asynchrony of recovery of excitability which has long been considered an important arrhythmogenic factor under various conditions, such as local myocardial ischemia, sympathetic activation, digitalis intoxication, etc[16,17]. Further studies to clear this point appear warranted.

An arrhythmia may also result from enhanced automaticity in ectopic pace-maker areas. A number of factors known to cause or potentiate development of ventricular arrhythmias can produce an increase in spontaneous firing of cells in the distal parts of atrioventricular node and in the His-Purkinje system[14,17]. It has been postulated that unbound FFAs might have detergent effect on cardiac cell membranes, thereby causing cation loss and resultant increase in ectopic impulse formation[18]. This assumption did not prove to be correct in our experiments in which after 60 min exposure to octanoate (2,4 mM), the spontaneous activity of ventricular strips completely (Figure 2). The initial, entirely regular spontaneous rate (about 70/min) was progressively reduced, and from time to time arrhythmias appeared. By the end of the test period, no activity was detected in 9 out 10 preparations. After 60 min washing with control solution, an almost complete recovery occurred. In this context, it is of interest to note that, in an unpublished series of experiments, octanoate (2,4 mM) did not alter substantially the intrinsic firing rate of the sinoatrial node of isolated rabbit atria[19].

In spite of the fact that in our experiments octanoate did not increase the automaticity in certain ectopic pacemaker areas of the normal cardiac tissue, it might still be shown to potentiate the effect of local myocardial ischaemia or catecholamines, both of which can cause spontaneous discharge of Purkinje fibres resulting in ventricular arrhythmias[14,17]. It is, of course, hardly to be expected that among the complex haemodynamic, autonomic, local and general metabolic changes occurring after acute coronary occlusion, the elevation of unbound FFA level would be alone responsible for the development of early post-infarction arrhythmias. In this respect, there is a real need for further studies of the cardiac electrophysiological effects of FFAs under in vivo conditions, with due regard also to the pathological processes characteristic of an acute myocardial infarction.

Whatever the role of a FFA may be as an additional factor in the genesis of arrhythmias due to a pathological situation, from our results with octanoate it seems reasonable to conclude that membrane function can be seriously altered by a FFA already in a normal Purkinje fibre. In favour of our suggestion, it has recently been shown that unbound FFAs (octanoate[10], oleate[20], and palmitate[20]) can produce severe arrhythmias, including ventricular fibrillation, even in normal isolated rat hearts.

Zusammenfassung. Am Beispiel von Octanoat (Kaprylsäure) wird nachgewiesen, dass Fettsäure das Aktionspotential von Purkinje-Fasern des Kalbes verändert. Dieselben Octanoatkonzentrationen von 2,4 mM unterdrückten auch die spontanen Aktivitäten isolierter Herzkammer-Streifenpräparate.

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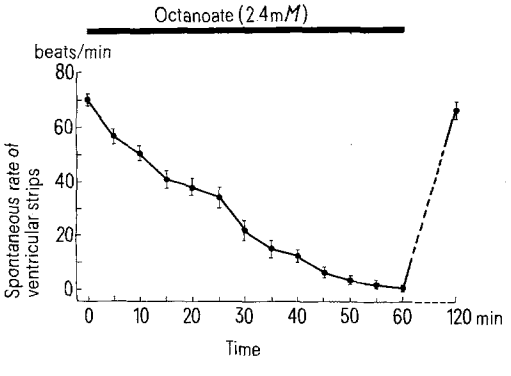

Fig. 2. Changes in the spontaneous rate of ventricular strips under the influence of octanoate. Exposure to octanoate started at 0 min right after the control measurements. Between 60 and 120 min, the preparations were washed with control solution. Vertical bars indicate ± S.E.; $n = 10$.

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Absence of Evidence of Biotransformation of Morphine to Codeine in Man

In studies of the human metabolism of morphine, it was found that in addition to morphine glucuronide, morphine ethereal sulfate, normorphine and normorphine conjugate were metabolites of morphine[1]. A recent paper[2] reporting the formation of codeine from morphine in man prompts the publication of the present paper.

Material and methods. Urine was collected for consecutive 24-hour periods from post-addict prisoner volunteers receiving chronic administration of morphine sulfate, 60 mg q.i.d., s.c., and was analysed for morphine and its metabolites with thin-layer chromatography (TLC)[3] and gas-liquid chromatography (GLC)[4]. Spots of morphine and