found to cause phase shifts in hamster locomotor activity rhythms, the magnitude and direction of which are dependent upon the time of administration in the animal's activity cycle[14]. Diazepam also appears to block light-induced phase advances, at doses that do not block light-induced phase delays, in hamsters free-running in constant dark[15]. Alternatively, phase delays, but not advances can be blocked by bicucculine, a GABA antagonist[16]. Microinjections of muscimol, a GABA agonist, when directed to the anterior hypothalamic suprachiasmatic nucleus (SCN), a putative circadian pacemaker, causes phase shifts in blinded hamsters which are similar in direction and magnitude to phase shifts induced by single injections of triazolam given to hamsters maintained in constant darkness[17]. The magnitude of the phase shifts induced by muscimol is dependent on the proximity of the microinjection to the SCN (Smith, Inouye and Turek, in press). In addition, phase advances induced by triazolam in the circadian rhythm of locomotor activity in golden hamsters can be abolished through lesions of the ventral lateral geniculate nucleus when this area includes the intergeniculate leaflet (IGL)[18]. The IGL sends afferent projections to the SCN, and is thought to be partially involved in the entrainment of activity rhythms to light/dark cycles[19]. These results suggest that the SCN may have a role in the regulation of GABA-mediated phase shifts of the circadian rhythm of locomotor activity. Taken together these data provide further evidence for a GABA-mediated system involved in the regulation or generation of the circadian rhythm of activity in the golden hamster.

Acknowledgments. We wish to thank Sue Losee-Olson and William Olson for technical assistance. This work was supported by NIH grant HD-09885, and the Upjohn Company.

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0014-4754/89/040334-04$1.50 + 0.20/0

Failure of opioids to affect excitation and contraction in isolated ventricular heart muscle

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Received 29 July 1988; accepted 1 December 1988

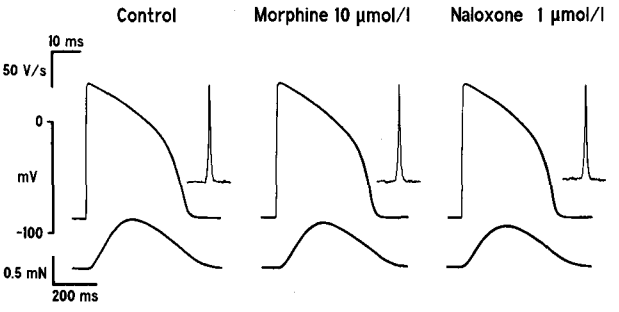
Summary. The opioid agonists morphine (selective for μ-receptors) and ethylketocyclazocine (selective for kappa-receptors), at concentrations evoking strong effects in neuronal structures, did not significantly affect the configuration of the intracellularly recorded action potential and the force of contraction in ventricular heart muscle isolated from guinea pigs, rabbits and man. These results suggest that any changes of heart functions in vivo in response to opioid-like drugs are probably not mediated postsynaptically at the myocardial cell membrane but rather presynaptically, influencing the release of noradrenaline and/or acetylcholine from the nerve terminals.
Key words. Heart muscle; opioids; morphine; ethylketocyclazocine; cardiac function; presynaptic modification.

Morphine and other opioid-like drugs have been reported to influence hemodynamic parameters in vivo[1, 2] and to depress cardiac functions in vitro[3–5]. The discovery of different subtypes of opioid receptors[6] and the evidence for the presence of enkephalins in the heart[7] have renewed the interest in the study of opioids in this tissue[8]. Whereas the earlier in vitro studies can be best explained by the existence of postsynaptic μ-receptors, it has been suggested more recently that the sympathetic axons innervating the sinus node of the rabbit possess

presynaptic opioid kappa- but not μ- or delta-receptors [9]. Similar results were obtained in guinea pig atrial noradrenergic nerves [10]. In preliminary studies in guinea pig left atria, we did not see any myocardial depressant effects of the delta-selective agonists leu-enkephalin and met-enkephalin or the sigma-selective agonist SKF 10,047 up to concentrations of 10 μmol/l. We therefore focused our interest on the effects of μ- and kappa-receptor activation, which was achieved by the addition of morphine and ethylketocyclazocine, respectively. We show here in different heart muscle preparations from mammalian species, including man, that opioids fail to influence cardiac function directly, which indicates that functional opioid receptors are not present postsynaptically in the heart or, at least, are of negligible significance.

Materials and methods. The preparations were obtained from freshly stunned guinea pigs and rabbits, and from human patients undergoing open heart surgery for mitral valve replacement (for details see Eckel et al. [11]). Right ventricular papillary muscles and strips of human left ventricular papillary muscles were electrically driven at 1 Hz (pulse duration 0.1 ms; voltage 10–15% above threshold) in Tyrode's solution (composition in mmol/l: NaCl, 136.9; KCl, 5.4; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; $CaCl_2$, 1.8; glucose, 5.6) bubbled with 95% O_2 and 5% CO_2 at 37 °C (pH 7.4). Action potentials were recorded intracellularly with conventional microelectrode techniques and evaluated for resting potential (RP), overshoot (OS), amplitude (APA), duration at 20% and 90% of repolarization, APD_{20} and APD_{90}, respectively. The first time derivative (dV/dt) of the action potential was obtained by electronic differentiation and evaluated for dV/dt_{max}. The force of contraction (F_c) was recorded isometrically via an inductive force displacement transducer and a carrier frequency preamplifier. All signals were initially stored on FM tape machines and, for evaluation, transferred from the tape via a digital transient recorder to an XY-recorder. The following drugs were used (sources in parentheses): morphine hydrochloride (Merck, Darmstadt, FRG); (±)-ethylketocyclazocine mesylate (gift of Sterling-Winthrop, Rensselaer, USA); naloxone hydrochloride (Endo Labs, New York, USA).

Results and discussion. Morphine in cumulatively increasing concentrations up to 10 μmol/l did not influence significantly the force of contraction or any action potential



Effects of morphine and naloxone on F_c, APD and dV/dt_{max} in isolated human ventricular heart muscle. Original records under control conditions, 30 min after the addition of morphine, and 30 min after the addition of naloxone (morphine still present). Stable microelectrode impalement during the course of the experiment (60 min). Any desensitization phenomena were excluded, since the effects of both morphine and morphine + naloxone were continuously observed for 30 min.

parameters in ventricular preparations from guinea pigs, rabbits or man. The figure shows the original recordings of action potential, first time derivative of the action potential and the force of contraction of a human heart muscle preparation under control conditions and 30 min after the addition of morphine 10 μmol/l. The modest reduction in the force of contraction is probably unrelated to an activation of morphine receptors, since further addition of naloxone 1 μmol/l (30 min) did not reverse this effect. Neither morphine nor naloxone decreased dV/dt_{max}, which is indicative of the absence of local anesthetic effects. In addition, resting potential, overshoot and duration of the action potential remained fairly constant under all conditions. Both morphine and naloxone are obviously devoid of direct myocardial membrane effects at these concentrations. The slight and continuous reduction in the force of contraction occurs also under control conditions and reflects a rundown phenomenon, which can be observed under any in vitro conditions; it varies quantitatively, depending on species, tissue, and experimental conditions.

Morphine 10 μmol/l and ethylketocyclazocine 10 μmol/l were equally ineffective in human papillary muscles. A summary of 5 and 2 experiments, respectively, is given in table 1. Similarly, neither morphine 10 μmol/l in rabbit papillary muscles nor ethylketocyclazocine 10 μmol/l in guinea pig papillary muscles were able to produce any significant changes of the action potential or the force of contraction in our experiments. A summary of 5 and 6 experiments, respectively, is given in table 2. An earlier

Table 1. Action potential parameters and force of contraction as influenced by morphine (10 μmol/l) and ethylketocyclazocine (EKC) (10 μmol/l) in human papillary muscles (means ± SEM)

		APA (mV)	RP (mV)	OS (mV)	APD_{20} (ms)	APD_{90} (ms)	dV/dt_{max} (V/s)	Fc (mN)
Control	(n=5)	114 ± 2	−84 ± 1	30 ± 1	167 ± 16	392 ± 23	128 ± 17	0.73 ± 0.35
Morphine	(n=5)	115 ± 2	−84 ± 1	31 ± 1	168 ± 14	383 ± 21	137 ± 19	0.67 ± 0.30
Control	(n=2)	122	−90	32	138	383	234	0.15
EKC	(n=2)	122	−90	32	127	360	224	0.14

Table 2. Action potential parameters and force of contraction as influenced by morphine (10 μmol/l) in rabbit papillary muscles and by ethylketocyclazocine (EKC) (10 μmol/l) in guinea pig papillary muscles (means ± SEM)

		APA (mV)	RP (mV)	OS (mV)	APD_{20} (ms)	APD_{90} (ms)	dV/dt_{max} (V/s)	Fc (mN)
Control	(n=5)	111 ± 3	−83 ± 1	26 ± 2	65 ± 5	165 ± 13	228 ± 33	0.68 ± 0.15
Morphine	(n=5)	111 ± 3	−83 ± 1	26 ± 3	66 ± 6	166 ± 12	235 ± 31	0.66 ± 0.15
Control	(n=6)	129 ± 1	−90 ± 0	39 ± 1	94 ± 5	175 ± 6	225 ± 28	0.1 ± 0.016
EKC	(n=6)	131 ± 2	−90 ± 0	41 ± 2	90 ± 6	174 ± 7	231 ± 30	0.09 ± 0.011

report[5] on the effects of morphine on rabbit papillary muscles has demonstrated a small negative inotropic effect which was eliminated upon the addition of naloxone 0.1 μmol/l. The morphine-induced negative inotropy was, however, not seen in the presence of atenolol 1 μmol/l, which led the authors to conclude that the effect of morphine on cardiac contractility was mediated by an indirect effect, located presynaptically at the adrenergic nerve terminals. This interpretation, however, is in conflict with the results of Starke et al.[9] and Fuder et al.[10], who have shown that the inhibition of noradrenaline release by opioids is mediated by kappa-receptors and not by μ-receptors. An alternative explanation for the results of Saxon et al.[5] would be an interaction between morphine and β-receptor activation affecting the adenylate cyclase. To test this possibility, we also investigated the interaction between morphine 10 μmol/l and isoprenaline 30 nmol/l. Under these conditions also, however, morphine failed to affect either the action potential or the force of contraction in human and guinea pig papillary muscles (not shown). We, therefore, favor the view that the action of the heart is not directly regulated by the release of endogenous opioids. This is unrelated to the possibility that endogenous opioids may significantly alter cardiac function by modulation of the release of noradrenaline[8-10] or acetylcholine[12-15].

Acknowledgments. We thank Dr H. Fuder (Pharmakologisches Institut der Universität Mainz) for valuable comments and suggestions. We also thank Prof. Satter (Abteilung für Herz-, Thorax- und Gefäßchirurgie der Chirurgischen Klinik der Johann Wolfgang Goethe-Universität Frankfurt) for supplying us with heart muscle preparations. H. N. and H. J. are supported by grants from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

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0014-4754/89/040337-03$1.50 + 0.20/0

Intramuscular comparison of myosin isozymes and light chains in rat extensor digitorum longus muscle

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Received 8 September 1988; accepted 12 January 1989

Summary. Complete muscle cross sections were obtained from the proximal and distal third regions of ten rat extensor digitorum longus muscles. Electrophoretic methods were then used to quantify the various myosin isozymes and light chains in each muscle specimen. The results demonstrated that the relative distribution of the various myosin isozyme and light chain variables do not vary significantly between the two sampling regions.
Key words. Myosin light chains; myosin isozymes; skeletal muscle.