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Response of Mammalian Cardiac Muscle to Certain Sympathomimetics in Presence of Morphine. (31244)

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It has long been accepted that exposure of the myocardium to therapeutic concentrations of morphine does not result in significant alterations in cardiac performance. The heart rate is either unaffected or slightly decreased. There appear to be no consistent effects on cardiac output or blood pressure, nor is the ECG altered. However, large doses apparently produce bradycardia (Goodman and Gilman(1); Drill(2)). Although therapeutic amounts of morphine thus appear to have little effect upon the myocardium of the intact animal, its beneficial action in patients with acute left ventricular failure, pulmonary edema, and paroxysmal hyperpnea associated with cyanotic congenital heart disease is well recognized. However, the mechanism(s) whereby morphine exerts these beneficial effects is not well understood. Wood(3) suggested that paroxysmal hyperpnea was due to obstructive spasm of the right ventricular infundibulum which others speculated might be the result of an increase in endogenous myocardial catecholamine release (Johnson(4) and Honey *et al*(5)); the implication being that morphine acts by inhibiting the positive inotropic action of catecholamines upon the myocardium.

Since the cardiovascular effects of morphine are complicated by its central as well as peripheral actions, we decided to test the hypothesis that morphine may antagonize the action of catecholamines upon the myocardium by employing *in vitro* techniques.

Methods. The cat papillary muscle preparation has previously been described in detail (Tanz(6)). Briefly, cats were sacrificed by cardiectomy under light ether anesthesia. Two papillary muscles from the right ventricle were quickly isolated with minimal trauma, mounted on muscle holders and placed in 2 chambers containing a modified Krebs-Ringer bicarbonate solution enriched with glucose. The perfusate was maintained at a constant temperature of 37.5°C and a mixture of 95% O_2 and 5% CO_2 bubbled through it. Tension for each muscle was adjusted so that stimulation would yield a maximal force of contraction. Stimulation was at suprathreshold voltages, with a frequency of 1/sec and a duration of one millisecond using separate Grass (Model S-4) stimulators. Isometric contractile force was recorded using the amplified output of Sanborn (fta 30) transducers which were monitored on a direct-writing Sanborn 2-channel recorder.

The following experimental groups were studied: a) untreated controls; b) morphine HCl (1 to 100 μg/ml); c) isoproterenol (IPNE) and epinephrine controls (0.2 μg/

TABLE I. Effect of Morphine on Contractile Force of the Cat Papillary Muscle Preparation (Expressed as Percent of Control Just Prior to Addition of Drug).

Substance	Conc (μg/ml)	N	Min after exposure 20′	40′	60′
Control		21	98	98	101
Morphine	1	16	96	92	85
"	3	14	96	91	89
"	10	15	96	91	87
"	30	6	95	97	92
"	100	13	100	91	80

ml); and d) the addition of morphine (1 to 100 μg/ml) followed one hour later by isoproterenol or epinephrine (0.2 μg/ml). Experimental groups were randomized, and each muscle was exposed only once to morphine. Results were expressed as the percent change in contractile force from the control reading obtained just prior to addition of a drug.

The isolated cat (Langendorff) heart preparation as modified by Anderson and Craver (7) was employed for simultaneous recording of contractile force, heart rate and coronary flow on an Offner Dynograph. Heart rate was monitored by sewing 2 fine silver wires into the auricles and recorded by means of an integrating heart rate monitor. A third wire, which served as the ground, was placed in the column of fluid perfusing the heart. Cardiac contractile force was recorded by tying a suture to the apex of the heart, passing it around a glass pulley and then to a Grass model FT .03 force displacement transducer. The perfusate was a modified Krebs-Ringer bicarbonate solution enriched with glucose, saturated with 95% O_2 and 5% CO_2, kept at a constant temperature of 37.5°C and continuously recycled. Heart rate was recorded as beats/minute, coronary flow as drops/unit time, and contractile force in terms of mm. For convenience and ease of comparison, all values were converted to percent of the control just prior to addition of a drug.

The previously noted parameters were monitored on the following experimental groups: a) untreated controls; b) morphine HCl (0.3 to 100 μg/ml); c) epinephrine (0.2 μg) and tyramine (25 μg/ml) controls; and d) the addition of morphine HCl (10 or 100 μg/ml) followed 20 minutes later by epinephrine or tyramine (as in c). Morphine was allowed to remain in the perfusate for 20 minutes at each concentration before epinephrine or tyramine was added. Each preparation was exposed to only one concentration of morphine and all experimental groups were randomized. In another experimental series the action of morphine (100 μg/ml) was compared to untreated controls over a 3-hour period.

Ventricular catecholamine analyses were performed on control and morphine-treated (100 μg/ml) isolated hearts one hour after mounting, as described previously (Tanz and Marcus(8)).

Results. A. The direct action of morphine on the heart is shown in Tables I and II, and Fig. 1. The addition of morphine HCl to the papillary muscle perfusate produced no significant alteration in contractile force for the first 20 minutes, regardless of concentration. However, at 40 minutes there is a definite indication that preparations exposed to morphine displayed a negative inotropic response, and this becomes clearly established by 60 minutes. The degree of diminution in contractile force did not appear to be dose-dependent. Immediately following the addition of 100 μg/ml a transient positive inotropic action was usually observed.

TABLE II. Effect of Morphine on the Cat Langendorff Preparation (Expressed as Percent of Control Just Prior to Addition of Drug).

Conc (μg/ml)	N	Contractile force 10′	20′	Heart rate 10′	20′	Coronary flow 10′	20′
Control	8	99.8	98.9	101.2	101.9	103.8	105.3
.3	4	96.5	92.5	93.3	90.5	97.5	88.0
1.0	4	108.0	101.3	103.3	101.5	103.0	98.0
3.0	4	106.5	96.5	95.5	94.3	107.8	105.5
10.0	15	100.8	95.9	97.7	97.4	102.9	101.9
30.0	4	104.3	93.8	100.5	92.8	98.3	82.5
100.0	15	99.3	95.2	108.9	100.9	108.3	101.8

Table II presents results obtained when morphine was added to the cat Langendorff perfusate and shows that in the presence of morphine contractile force declined somewhat more rapidly than in the untreated controls. In general, this same pattern was also found but to a lesser degree with heart rate and coronary flow. But once again, there did not appear to be any dose-dependency.

The effects of morphine exposure over a 3-hour period in the cat Langendorff preparation are illustrated in Fig. 1, which shows a steady diminution in both contractile force and heart rate.

Although morphine apparently depresses the myocardium to a greater or lesser extent, depending on duration of exposure, no significant difference was found in the ventricular catecholamine content between those isolated hearts exposed to 100 μg/ml and untreated hearts at the end of one hour. The ventricular norepinephrine content of 6 controls was 1.45 μg/g (S.E. $= \pm$ 0.14) and for 7 morphine-treated hearts it was 1.40 μg/g (S.E. $= \pm$ 0.19), the "p" value being $>.50$ between the two groups. The control level of 1.45 μg/g is slightly less than 1.63 μg/g reported previously for freshly excised cat ventricles

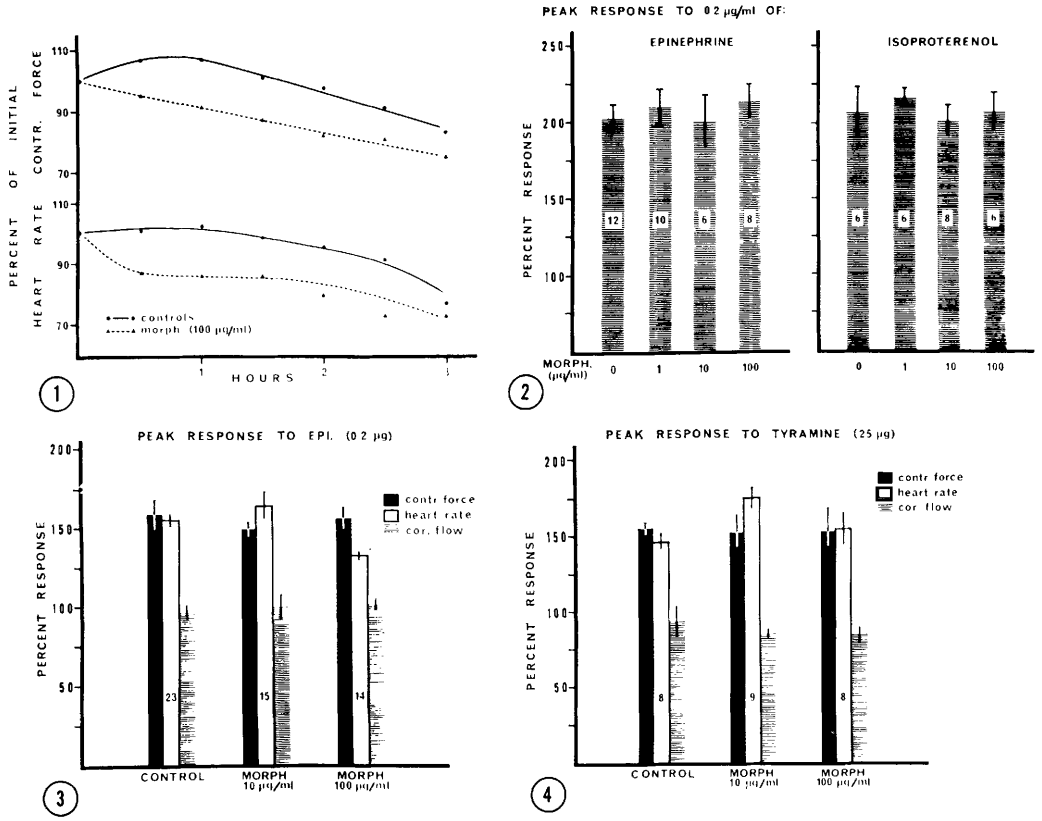

FIG. 1. Response of the whole isolated heart preparation to morphine HCl (100 μg/ml). Solid curves = untreated controls(8); dotted curves = morphine-treated(5). Morphine given at +2 min.

FIG. 2. Peak response of the papillary muscle preparation to epinephrine and isoproterenol, after 1 hr exposure to morphine. (Figures in bars represent number of individual experiments; vertical lines represent calculated standard error.)

FIG. 3. Peak response of the whole isolated heart preparation to epinephrine, after 20 min exposure to morphine. (Figures in bars represent number of individual experiments; vertical lines represent calculated standard error.)

FIG. 4. Peak response of the whole isolated heart preparation to tyramine after 20 min exposure to morphine. (Figures in bars represent number of individual experiments; vertical lines represent calculated standard error.)

(Tanz and Marcus(8)), which is to be expected since isolated hearts are devoid of any sympathetic nerve supply. These data agree surprisingly well with that of Campos *et al* (9) who reported cat left ventricular total catecholamine levels of 1.61 and 1.42 μg/g at 0 and 60 minutes, respectively, after perfusion.

B. The action of various sympathomimetics upon morphine-treated cardiac preparations is illustrated in Fig. 2-4. Fig. 2 shows the peak responses elicited by epinephrine and isoproterenol on the papillary muscle preparation. Similarly, the peak responses following epinephrine and tyramine on the morphine-treated isolated heart preparation are illustrated in Fig. 3 and 4, respectively. Although minor exceptions occur, in general the data show that prior exposure to morphine did not alter the degree of activity elicited by the subsequent administration of epinephrine, isoproterenol or tyramine.

Discussion. These results demonstrate that exposure of the cat papillary muscle and whole isolated heart preparations to morphine (0.3-100 μg/ml) produces a slight negative inotropic response, and in the isolated heart preparation little or no decrease in heart rate and coronary flow. The data suggest that within the range of concentrations studied, degree of myocardial depression is apparently unrelated to concentration of morphine. When exposed to 100 μg/ml of morphine, the whole isolated heart preparation displayed a steady decline in both contractile force and rate, in comparison to untreated controls. Thus, over a wide range of concentrations morphine directly depresses myocardial performance of *in vitro* cat cardiac preparations to varying degrees, which probably depends on the duration of exposure.

The results obtained do not agree with those reported by Gruber and Robinson(10) who noted that "small to moderate" doses of morphine increased cardiac contractile force, rate and coronary flow in isolated turtle, rabbit and cat hearts. In their studies larger doses, however, did produce myocardial depression. The cardiac depression we observed confirms the study by Schmidt and Livingston(11) who used concentrations of morphine up to 200 μg/ml on isolated guinea pig, cat and dog heart preparations. It should be pointed out, however, that although the isolated heart may be depressed by morphine the heart *in situ* is not significantly affected by doses sufficient to cause a marked fall in blood pressure (Schmidt and Livingston(11); Sollmann(12)). Vasko *et al*(13) have presented data indicating that cardiac performance is actually augmented in intact dogs, although this could be abolished by beta adrenergic blockade or adrenalectomy. They concluded that the positive inotropic effect of morphine was indirect, the result of sympathoadrenal discharge.

After administration of a single non-convulsant dose of morphine (100 mg/kg), Klingman and Maynert(14) reported a significant increase in rat cardiac catecholamine levels, as did Stitzel *et al*(15) who observed an increase in rabbit cardiac catecholamine content 5 hours following 50 mg/kg of morphine. Moreover, these latter workers showed that catecholamine content of intact adrenal glands is significantly reduced following morphine (150 mg/kg), which suggests that the increase in cardiac catecholamines was due to their release from the adrenals and subsequent accumulation in the heart. The demonstration that morphine-induced hyperglycemia is greatly modified following adrenalectomy appears to confirm the validity of this belief (Vassalle(16)). Nevertheless, chronic administration of morphine is characterized by a diminution in cardiac catecholamine levels, which apparently is not due to the impairment of norepinephrine synthesis (Klingman and Maynert(14)). Thus the proposal that morphine inhibits biogenic amine synthesis (*e.g.*, reserpine-like activity), does not seem likely.

The lack of any statistically significant difference in ventricular catecholamine content between untreated and morphine-treated isolated hearts, suggests that if any alteration in content is to be observed, morphine would probably have to be administered solely to intact animals.

We were specifically interested in testing the hypothesis that morphine may inhibit the action of sympathomimetic agents on the

heart. Since morphine did not alter cardiac catecholamine levels, it was not surprising to find that isolated cardiac preparations failed to show any consistently different responses to either epinephrine, isoproterenol or tyramine in the presence or absence of morphine. It is possible that the beneficial results attributed to morphine in certain clinical conditions (see Introduction), may be the result of its central nervous system activity whereby catecholamines are released and peripheral levels eventually reduced, or by mechanisms unrelated to catecholamines (Guntheroth *et al*(17)). A likely possibility may reside in the explanation provided by Vasko and co-workers(13), who demonstrated that morphine markedly diminishes total peripheral resistance resulting in increased peripheral vascular capacitance and an overall improvement in hemodynamics. Their findings of augmented ventricular performance with morphine in the intact animal are in obvious conflict with earlier speculation that morphine would reduce "spasm" of the right ventricular infundibulum(3,4,5).

Summary. The action of morphine upon isolated mammalian cardiac preparations was reevaluated in order to test the hypothesis that morphine has the ability to antagonize the action of catecholamines on the heart. Over a wide range of concentrations (0.3-100 μg/ml) morphine slightly depressed contractile force, heart rate and coronary flow. Following exposure of the whole isolated heart preparation to morphine (100 μg/ml) for one hour, ventricular catecholamine levels were similar to their untreated controls. Furthermore, no consistent alterations in physiological response to epinephrine, isoproterenol or tyramine could be correlated with the presence or absence of morphine regardless of its concentration in the perfusate. These data fail to confirm the suggestion that morphine acts as a sympatholytic on the heart.

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Effect of Dietary Iodine Upon Egg Production, Fertility and Hatchability.*† (31245)

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Two recent reports(1,2) have described the toxic effects of excess dietary iodine upon rats and rabbits. The primary effect, when fed to pregnant rats, was a failure of lactation and high mortality of the young. Dietary levels of iodine from 500 to 2500 ppm as KI caused

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