THE MODIFICATION OF INOTROPIC ACTION OF OUABAIN BY 6-HYDROXYDOPAMINE AND α -METHYL-P-TYROSINE IN DOGS

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Abstract—The action of ouabain on myocardial inotropism pretreated with 6-hydroxydopamine and α -methyl-p-tyrosine was studied. Inotropic action of ouabain was not changed by depletion of catecholamines in the brain, in which the central sympathetic neurons were destroyed by an intraventricular administration of 6-hydroxydopamine. Systemic administration of 6-hydroxydopamine and α -methyl-p-tyrosine reduced ventricular catecholamines to 5.3% and 20.8% of the control, respectively. Percent increase of contractility by ouabain after the pretreatment of 6-hydroxydopamine and α -methyl-p-tyrosine was reduced to 16.7% and 50.2% of the control, respectively. The results obtained suggest that catecholamines in the myocardium play some important role in producing cardiotonic action of cardiac glycosides. Brain catecholamines or the central sympathetic nervous system do not appear to participate in the exertion of the positive inotropic action of cardiac glycosides.

It was reported that the contractility of heart muscle may be modified by levels of myocardial catecholamines (1, 2).

Lee and Shideman considered that endogenous catecholamines in heart muscles serve as humoral agents for maintaining normal contractility because depletion of myocardial catecholamines by surgical sympathectomy or reserpine results in a depression of cardiac contractility. Tanz and Marcus suggested that the mechanism of the positive inotropic action of cardiac glycosides might be related at least in part to its ability to release endogenous catecholamines (2, 3, 4).

On the other hand, Spann et al. reported that pretreatment of reserpine interferes slightly with the production of maximum ouabain-induced augmentation but papillary muscles from cats with cardiac catecholamine depletion produced by chronic cardiac denervation displayed the same degree of augmentation in response to ouabain as did the untreated controls (5, 6).

The view that the release of catecholamines may be involved in the inotropic effect of ouabain is based mainly on the fact that the reserpine-treated heart shows an attenuated positive inotropic response to cardiac glycosides especially in isolated tissues (2, 3, 7, 8).

It has been noted that reserpine has direct depressant effects on the myocardium. Attenuation of glycoside action following reserpine may therefore result from effects unrelated to its catecholamine depleting action (9, 10, 11, 12).

A chemical sympathectomy can be done using 6-hydroxydopamine (13, 14).

α -Methyl-p-tyrosine also depletes the adrenergic transmitter by inhibiting norepinephrine synthesis (15).

The present experiments were designed to observe the role of central and peripheral catecholamines in the inotropic action of ouabain.

MATERIALS AND METHODS

Experiments were performed on the following four groups of mongrel dogs.

Group I: Non-treated dogs (control).

Group II (central sympathectomy): Twenty mg of 6-hydroxydopamine (6-OHDA) was administered into the lateral ventricles for the destruction of central sympathetic neurons. A stereotaxic apparatus was used for the administration into the lateral ventricles. Experiments were performed from 10 to 14 days after the above operation.

Group III (systemic sympathectomy): 6-OHDA was injected intravenously for the chemical sympathectomy of the peripheral nervous system. 6-OHDA was administered from 4 to 6 times at 2 or 3 day intervals. The total doses amounted to 35 mg/kg. Experiments were performed 2 days after the last administration.

Group IV (systemic administration of α -MT): α -Methyl-p-tyrosine (α -MT) as an enzyme blocking agent for catecholamine synthesis was injected intraperitoneally at a dose of 250 mg/kg to decrease the endogenous catecholamines in the myocardium and brain. Two thirds of the total amount of α -MT was injected 40 hr before and one third was injected 16 hr before experiments, respectively.

Adult mongrel dogs (8–14 kg) of either sex were prepared under pentobarbital anesthesia (30 mg/kg, I.V.) with artificial respiration. Ouabain was infused constantly into the femoral vein at a dose of 50 μ g/ml/3 min.

The contractile force of the myocardium was measured by a strain-gauge arch which was attached to the left ventricle parallel with lines of superficial myocardial fibers. Catecholamines in tissues were measured chemically by using fluorometric technique (16).

Catecholamine content in tissues is shown as total amounts of epinephrine and norepinephrine. Student t-test was used for a statistical analyses of data.

RESULTS

Group I: Mean maximum increase of contractility by ouabain was 61.5% of that before the infusion of ouabain in non-treated dogs. The catecholamine contents of cortex and brainstem were 0.089 μ g/g and 0.141 μ g/g, respectively. The catecholamine content of the myocardium was 1.446 μ g/g.

Group II: Chemical sympathectomy of central sympathetic neurons by 6-OHDA did not change the inotropic effect of ouabain whereas brain catecholamine content was reduced. Mean maximum increase of contractility was 50.9%, not a significant change compared to 61.5% in non-treated dogs (Table 2). Intraventricular administration of 6-OHDA reduced the catecholamine content of cortex and brainstem to 0.015 μ g/g and 0.032 μ g/g, respectively

TABLE 1. Total catecholamine levels of various tissues in control dogs, central sympathectomized dogs by 6-OHDA and systemic sympathectomized dogs by 6-OHDA and α -MT

(): number of animals				
TREATMENT	CONTROL	6-OHDA (L.V.)	6-OHDA (I.V.)	α -MT
TISSUE	(mean $\pm$ S.E.)	(mean $\pm$ S.E.)	(mean $\pm$ S.E.)	(mean $\pm$ S.E.)
CORTEX	0.089 $\pm$ 0.029 (7)	0.015 $\pm$ 0.002* (5)	0.062 $\pm$ 0.017 (6)	0.029 $\pm$ 0.005** (6)
BRAINSTEM	0.141 $\pm$ 0.044 (7)	0.032 $\pm$ 0.003* (5)	0.210 $\pm$ 0.046 (6)	0.130 $\pm$ 0.032 (6)
HEART	1.446 $\pm$ 0.192 (7)	1.032 $\pm$ 0.088 (5)	0.076 $\pm$ 0.017* (7)	0.301 $\pm$ 0.062* (7)

(μ g/g tissue wet weight)*: Significantly different from control ($P < 0.01$)**: Significantly different from control ($P < 0.05$)

6-OHDA (L.V.): Central sympathectomized dogs with 6-hydroxydopamine

6-OHDA (I.V.): Systemic sympathectomized dogs with 6-hydroxydopamine

 α -MT: Systemic sympathectomized dogs with α -methyl-p-tyrosineTABLE 2. Percent increase of peak contractile force by ouabain in control dogs, central sympathectomized dogs by 6-OHDA and systemic sympathectomized dogs by 6-OHDA and α -MT

N: number of animals				
	CONTROL	6-OHDA (L.V.)	6-OHDA (I.V.)	α -MT
	[N=7]	[N=10]	[N=7]	[N=8]
% increase of peak contractile force (mean $\pm$ S.E.)	61.5 $\pm$ 7.3 (100%)	50.9 $\pm$ 8.7 (82.7%)	10.3 $\pm$ 4.2* (16.7%)	30.9 $\pm$ 8.7** (50.2%)

*: Significantly different from control ($P < 0.01$)**: Significantly different from control ($P < 0.05$)

(): % of contractile force increase compared with control

6-OHDA (L.V.): Central sympathectomized dogs with 6-hydroxydopamine

6-OHDA (I.V.): Systemic sympathectomized dogs with 6-hydroxydopamine

 α -MT: Systemic sympathectomized dogs with α -methyl-p-tyrosine

(Table 1). Myocardial catecholamine content was 1.032 μ g/g which is only a slightly significant change as compared with the control.

Group III: In dogs treated with 6-OHDA intravenously, the inotropic action of ouabain was reduced in parallel with reduction of the myocardial catecholamine content. Mean maximum increase of contractility was 10.3% of that before the infusion of ouabain which corresponds to 16.7% of the control (Table 2). Myocardial catecholamine content was reduced to 0.076 μ g/g corresponding to 5.3% of the control. No significant change of brain catecholamine content was observed as the blood brain barrier prevents passage of 6-OHDA.

Group IV: Depression of the inotropic action of ouabain was also observed in the pretreatment of α -MT. Mean maximum increase of contractility was 30.9% of that before the infusion of ouabain which corresponds to 50.2% of the control (Table 2).

α -MT pretreatment reduced catecholamine content of myocardium and cortex to 0.301 $\mu\text{g/g}$ and 0.029 $\mu\text{g/g}$, respectively. There was however, no significant decrease of catecholamine content in the brainstem (Table 1).

The magnitude of depression of ouabain-induced inotropism in α -MT pretreated dogs was about one third of that in dogs treated with 6-OHDA intravenously. The degree of α -MT-induced decrease of myocardial catecholamine content was about one fourth of that by 6-OHDA administered intravenously.

Thus, ouabain-induced inotropism in α -MT pretreatment was depressed less than that in intravenous treatment with 6-OHDA. Such is in parallel with a lesser degree of myocardial catecholamine decrease by α -MT than that by 6-OHDA administered intravenously.

DISCUSSION

No definite conclusion has been reached concerning the role of cardiac norepinephrine stores on the inotropism of digitalis.

Lee and Shideman suggested that myocardial catecholamine stores serve as humoral agents for maintaining the normal contractility in the myocardium (1).

Tanz and Marcus observed that the augmentation of isometric tension produced by ouabain in isolated cat papillary muscles in which cardiac norepinephrine-depletion has been produced by guanethidine was substantially less than that of the controls (2). Tanz also found that pretreatment of a nontoxic concentration of dichloroisoproterenol, a beta-blocking agent, significantly reduced the magnitude of the positive inotropic action of ouabain (4). He suggested therefore that endogenous cardiac catecholamine stores may play an important role on the full response to cardiac glycosides.

Conversely, Spann and his associates reported that length-tension curves and force-velocity relations are not depressed in norepinephrine-depleted cat papillary muscles produced by chronic cardiac denervation (5). They also showed that cardiac norepinephrine stores and neural integrity are not essential for the positive effect of strophanthidin in length-tension curves and force-velocity relations of cat papillary muscles (6). Morrow and his co-workers reported that the positive inotropic actions of ouabain in strain-gauge arch which is sutured on the right ventricle are independent of autonomic innervation and myocardial catecholamine stores (17). Koch-Weser reported that the dose of propranolol which do not alter the basal contractility of isolated kitten papillary muscles has no effect on cumulative inotropic concentration-effect curves for ouabain, acetylstrophanthidin and digoxin (18).

The present investigations were undertaken to re-examine the involvement of central and peripheral endogenous catecholamines in digitalis inotropism by using new experimental agents, 6-OHDA and α -MT for central and peripheral chemical sympathectomy.

Central catecholamine depletion by 6-OHDA administered into the lateral ventricles had no influence on the ouabain-induced augmentation of contractility. Such an observation indicates that brain catecholamines or central sympathetic neurons do not participate in the positive inotropic action of glycosides.

The present studies revealed the depression in the contractility to ouabain in strain-gauge arch following peripheral chemical sympathectomy by intravenous administration of 6-OHDA. Similar results were obtained following the pretreatment of α -MT. The magnitude of depression by 6-OHDA in myocardial contractility was about 3 times of that by α -MT. The magnitude of depression in contractility more or less corresponded to the magnitude of myocardial catecholamine depletion as the myocardial catecholamine content following 6-OHDA was about 4 times less than that by α -MT.

These results are in accordance with those of Tanz who showed that the response to ouabain in catecholamine depleted preparations was significantly impaired when compared to that in the controls (2, 3, 4). Nevertheless, our observations do not parallel those of Spann et al. and Morrow et al. who confirmed that myocardial catecholamine stores have little involvement in the positive inotropic action of cardiac glycosides. We cannot explain this discrepancy. The possibility has to be considered that 6-OHDA and α -MT may interfere with the positive inotropic action of ouabain not by the adrenergic intervention but through other pharmacological actions not yet elucidated.

We sutured the strain-gauge arch on the left ventricle and in such a case, contractility is very much influenced by arterial pressure, heart rate and cardiac output. Under such a condition, the influence of autonomic innervation on the intrinsic contractile state and on the increment of contractility by cardiac glycosides has to be considered. The results obtained from our experiments by strain-gauge arch *in vivo* suggest that myocardial catecholamine levels are involved in producing the full positive inotropism of glycosides.

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