SUMMARY Serotonin has been found in the heart. Because cardiac serotonin is a potential endogenous source of inotropic support, an understanding of its metabolism in normal and failing hearts may be important. Cardiac serotonin was assayed in cardiomyopathic hamsters and their controls. Hearts were flushed free of blood vessels. Cardiac serotonin stores were 30–40% of those of norepinephrine. They were not affected by repeated injections of a mast cell granule depletor (H48/80) or the neurotoxins, 6-hydroxydopamine and 5,7-dihydroxytryptamine. Thus, cardiac serotonin appeared to be extraneural and not secondary to mast cell or platelet contamination. Inhibition of serotonin synthesis resulted in a prompt decrease, and inhibition of serotonin degradation led to a rapid increase in cardiac serotonin stores, demonstrating actual serotonin synthesis within the heart. Serotonin content (0.45 ± 0.012 μg/g in controls vs. 0.24 ± 0.009 μg/g in failing myopathic hearts) and synthesis (0.71 ± 0.016 pg/g per hour in controls vs. 0.028 ± 0.011 pg/g per hour in failing myopathic hearts) were significantly reduced in heart failure. Serotonin stores of uterus (a “control” organ) were identical for both strains. There was no difference in cardiac serotonin between the two strains in young hamsters. Human papillary muscles, taken at cardiac surgery, had serotonin levels (0.388 ± 0.027 pg/g) comparable to that found in hamster hearts. Thus, there are significant stores of serotonin synthesized within the heart. Both the stores and synthesis of serotonin are reduced in the failing myopathic hamster heart.

SEROPTONIN has been shown to exert a direct positive inotropic effect on mammalian myocardium (Buccino et al., 1967, Benfey et al., 1974). Recent studies have demonstrated the presence of this indolealkylamine in the heart and blood vessels of rats, cats, and dogs (Beauvallet et al., 1968; Berkowitz et al., 1974; Votavova et al., 1971; Madan et al., 1970). Though cardiac serotonin, like cardiac norepinephrine, appears to contribute little to basal myocardial contractility (Buccino et al., 1967), the presence of serotonin in the heart suggests it may be an endogenous source of inotropic support during physiological or pathological cardiac stress. Alterations in serotonin metabolism also have been associated with cardiac injury (Spatz, 1969; Crawford, 1963). Therefore, we decided to characterize the metabolism of cardiac serotonin and to determine whether cardiac stores of this compound were affected in a natural model of heart disease—the cardiomyopathic Syrian hamster (Gertz, 1972).

Methods

The dystrophic Syrian hamster is a useful, reproducible, spontaneous model for human myocardial disease (Gertz, 1972). Female cardiomyopathic (Bio 53.58) and matched control (Bio R.B.) Syrian hamsters, 90–110 and 240–270 days old, were used in these experiments. The former represented an early stage of the cardiomyopathy and the latter represented the stage of cardiac decompensation and failure. All hamsters were allowed at least 2 weeks to acclimate to our laboratory animal facility after delivery from the breeder (TELACO). A 12-hour light, 12-hour dark cycle was maintained in the animal housing area. Hamsters were allowed water but deprived of food (Purina rat chow) for 24 hours preceding the experiment.

The hamsters were killed by decapitation between 10 a.m. and 2 p.m. The hearts, in situ, were flushed free of blood with 20 ml of heparinized saline, administered by retrograde perfusion under

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pressure through the aorta by hypodermic syringe; the perfusate drained through a right ventricular and a right atrial vent. The hearts then were removed, opened, and rinsed; the ventricles were dissected free of atria and great vessels and then immediately frozen on dry ice. The frozen tissues were weighed and homogenized with a Polytron homogenizer in 10 volumes of acidified butanol (0.85 mg of concentrated hydrochloric acid per liter of N-butanol). Serotonin and 5-hydroxyindoleacetic acid (5-HIAA) were measured spectrofluorometrically (Curzon and Green, 1970). Tryptophan was measured by the method of Denckla and Dewey (1967). The tissue catecholamines were assayed by the method of Sole and Hussain (1977).

Pargyline hydrochloride, α-propyldopacetamide, 6-hydroxydopamine, and 5,7-dihydroxytryptamine were obtained from Regis Chemical Co. Compound H4/80 was obtained from Sigma Chemicals.

A minimum of six hamsters in each of the control and cardiomyopathic groups was used for each experiment. Results are expressed as means ± SEM. The statistical significance of differences between groups was determined by Student’s t-test.

Results

We established the presence of significant stores of serotonin in the normal hamster heart (Table 1). It was possible that these stores were localized largely in mast cells present within the myocardium. Thus, we administered 1 mg/kg of compound H4/80, an effective depletor of mast cell histamine and serotonin (Snyder et al., 1964), to hamsters by intraperitoneal injection every 8 hours for 3 days. The hamsters were killed 18 hours after the last dose. Cardiac serotonin was unaffected by H4/80 administration (Table 1).

We then wished to determine whether serotonin actually is synthesized within the heart or merely accumulated in mast cells present within the myocardium. Thus, we administered 1 mg/kg of compound H4/80, an effective depletor of mast cell histamine and serotonin (Snyder et al., 1964), to hamsters by intraperitoneal injection every 8 hours for 3 days. The hamsters were killed 18 hours after the last dose. Cardiac serotonin was unaffected by H4/80 administration (Table 1).

We next performed experiments to determine whether cardiac serotonin was stored in sympathetic nerve endings, in actual serotonergic nerves, or in extraneural loci in the myocardium. We destroyed the cardiac noradrenergic sympathetic nerve endings in one group of hamsters by administering to them 6-hydroxydopamine (Votavova et al., 1971). Another group of hamsters received 5,7-dihydroxytryptamine, a compound which is neurotoxic to both noradrenergic and serotonergic nerve endings (Creveling et al., 1975). Administration of either neurotoxin resulted in a marked loss of cardiac catecholamine stores; however, those of serotonin were unaffected (Fig. 1).

To determine whether cardiac serotonin was present in, and, hence, relevant to, the human heart, papillary muscles were taken from the hearts of 12 patients undergoing mitral valve replacement. During cardiac surgery, these hearts were perfused with a cold cardioplegic solution, minimizing platelet contamination of our specimens. We found serotonin, 0.388 ± 0.027 μg/g, in these papillary muscle specimens, a value comparable to that seen in the hamster. Institutional rules pertaining to human subjects were complied with in performing this study.

As cardiac serotonin appeared to be a potential endogenous source of inotropic support for the failing myocardium, an understanding of its metabolism during cardiac decompensation and failure may be important. Thus, we examined cardiac serotonin concentration and content during both the early and late states of hamster cardiomyopathy. We found no significant difference in cardiac serotonin between young myopathic hamsters and their controls (Table 2). During the stage of cardiac decompensation, both the content and concentration of cardiac serotonin fell in the myopathic hamsters (Table 2). We also examined uterine serotonin stores to determine whether our observations were

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### Table 1 Serotonin Concentration in Normal Hamster Hearts

<table>
<thead>
<tr>
<th></th>
<th>Serotonin concentration (μg/g)</th>
<th>5-HIAA concentration (μg/g)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.486 ± 0.013</td>
<td>0.064 ± 0.002</td>
</tr>
<tr>
<td>Saline vehicle</td>
<td>0.485 ± 0.005</td>
<td>0.059 ± 0.003</td>
</tr>
<tr>
<td>Compound 48/80</td>
<td>0.485 ± 0.010</td>
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</tr>
<tr>
<td>α-Propyldopacetamide</td>
<td>0.378 ± 0.013*</td>
<td></td>
</tr>
<tr>
<td>Pargyline</td>
<td>0.610 ± 0.018*</td>
<td>0.041 ± 0.005†</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.
* Differs from control and vehicle injected at P < 0.001.
† Differs from control and vehicle injected at P < 0.01.
Figure 1: Effect of neurotoxin administration on cardiac norepinephrine and serotonin contents in hamsters. On day 1, each received propranolol (5 mg/kg), phenoxybenzamine (10 mg/kg), and methysergide (1 mg/kg) by intraperitoneal injection. Fifteen minutes later, they received either 1 μg/g of vehicle [ascorbic acid (6 mg/ml) in degassed saline made to 10⁻³ N HCl] or 6-hydroxydopamine hydrobromide (75 mg/kg), or 5,7-dihydroxytryptamine creatinine sulfate (110 mg/kg) in vehicle. Vehicle or neurotoxin administration (without the amine blockers) was repeated on day 3 and at double the dose on days 8 and 10. The hamsters were killed and their ventricles taken for assay on day 15. Each value is the mean ± SEM for eight hamster hearts. *Significantly different from control at P < 0.001.

Discussion

Serotonin has been identified in the hearts of rats (Beauvallet et al., 1968, Berkowitz et al., 1974), cats (Votavova et al., 1971), and dogs (Madan et al., 1970). In this communication, we demonstrate significant endogenous stores of serotonin in both the hamster and human heart. In addition, we now describe some of the characteristics and metabolism of these stores.

The serotonin concentration of the hamster heart is 40-50% of that determined for cardiac norepinephrine (Fig. 1). Serotonin may be accumulated and stored by both platelets (Morrissey et al., 1977, Austen and Humphrey, 1963) and mast cells (Austen and Humphrey, 1963). We thoroughly flushed and rinsed the hearts prior to assay to minimize platelet contamination. Furthermore, the relatively high concentration of cardiac serotonin would seem to obviate an artifact due to platelet or mast cell contamination. It should be noted that serotonin is merely a manifestation of a general alteration in peripheral serotonin metabolism. No change in uterine serotonin was found (Table 2). We had shown previously that the development of heart failure in the cardiomyopathic hamster is associated with an increase in cardiac sympathetic tone (Sole et al., 1975). We also described a reduction in the norepinephrine stores of the failing hamster heart that was secondary to this increase in sympathetic activity (Sole et al., 1975). We were able to restore cardiac norepinephrine content completely to normal by the administration of the peripheral ganglionic blocker, chlorisondamine. Administration of chlorisondamine (10 mg/kg, ip, every 6 hours for 24 hours) to myopathic hamsters did not affect the reduced serotonin content of the failing heart (0.360 ± 0.010 μg/g in untreated vs. 0.347 ± 0.013 μg/g in treated).

We studied the synthesis of cardiac serotonin in failing hamsters and their controls by examining the accumulation of serotonin in the heart, 1 hour after the administration of pargyline (75 mg/kg, ip) (Neff and Tozer, 1968). Serotonin synthesis appeared significantly reduced in the decompensating myopathic hearts (Table 3). As serotonin synthesis, at least in the brain, is dependent on the availability of tryptophan (Fernstrom and Wurtman, 1971), the decline in cardiac serotonin suggested a possible alteration in precursor tryptophan. Both groups of hamsters exhibited similar plasma tryptophan (12.97 ± 1.04 μg/ml in controls vs. 12.77 ± 0.54 μg/ml in failing) and cardiac tryptophan (10.92 ± 1.54 μg/g in controls vs. 9.30 ± 0.55 μg/g in failing) concentrations.

Table 2: Serotonin Stores in Hearts and Uteri of Control and Myopathic Hamsters

<table>
<thead>
<tr>
<th></th>
<th>Serotonin concentration (μg/heart)</th>
<th>Serotonin content (μg/heart)</th>
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<tbody>
<tr>
<td><strong>Hearts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (90-110 days old)</td>
<td>0.490 ± 0.018</td>
<td>0.148 ± 0.009</td>
</tr>
<tr>
<td>Myopathic</td>
<td>0.486 ± 0.024</td>
<td>0.136 ± 0.003</td>
</tr>
<tr>
<td>Control (240-270 days old)</td>
<td>0.484 ± 0.007</td>
<td>0.206 ± 0.009</td>
</tr>
<tr>
<td>Myopathic</td>
<td>0.352 ± 0.013*</td>
<td>0.158 ± 0.007*</td>
</tr>
<tr>
<td><strong>Uteri</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (240-270 days old)</td>
<td>0.319 ± 0.015</td>
<td>0.089 ± 0.006</td>
</tr>
<tr>
<td>Myopathic</td>
<td>0.296 ± 0.010</td>
<td>0.108 ± 0.007</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

* Differs from age-matched control at P < 0.001.
not stored in the mast cells of man (Austen and Humphrey, 1963); thus, mast cell contamination could not account for the serotonin present in human papillary muscle. In an earlier study, Snyder and co-workers (1964) labeled rat hearts with exogenously administered \(^{14}C\)-serotonin. A rigorous injection schedule of H48/80, a depletor of mast cell serotonin, failed to deplete the label from the hearts. We did not alter endogenous cardiac stores of serotonin using a similar injection schedule. Although both platelets and mast cells are avid repositories for free circulating serotonin, they do not synthesize this compound (Morrissey et al., 1977). The relatively prompt decrease in cardiac serotonin, following the inhibition of tryptophan hydroxylase, and the corresponding increase, following the inhibition of monoamine oxidase, suggest that the amine is actually synthesized within the heart. The substantive increase in serotonin concentration after monoamine oxidase inhibition and the presence of cardiac 5-HIAA suggest also that monoamine oxidase catalyzes a major pathway for the metabolism of the heart's serotonin stores. Thus, these experiments would seem to establish that the presence of serotonin in the heart is not merely reflective of stores sequestered in intramyocardial mast cells or platelets. Furthermore, the indolealkylamine appears to be both synthesized and metabolized within the heart.

Serotonin may be taken up by noradrenergic nerve endings (Fillion et al., 1971); peripheral serotonergic neurons also have been described recently (Gershon et al., 1977). Monoaminergic nerve endings can be destroyed selectively by the neurotoxins, 6-hydroxydopamine and 5,7-dihydroxytryptamine. The former is relatively specific for catecholaminergic nerves (Votavova et al., 1971), whereas the latter destroys both catecholaminergic and serotonergic nerve endings (Creveling et al., 1975). Hearts were depleted of their catecholamine stores after administration of either neurotoxin to the hamsters; cardiac serotonin, however, was not affected. Thus, cardiac serotonin does not appear to be associated with either sympathetic or serotonergic nerve endings. The possible association of cardiac serotonin stores with myocardial inclusion bodies (Page, 1967) is of obvious interest.

The physiological significance of cardiac serotonin remains speculative. The vascular effects of this amine are well known. In the heart, it is a direct coronary vasodilator (Mena and Vidrio, 1976), suggesting that perhaps these myocardial stores play a role in the mediation of intramyocardial vascular tone. Other biological properties appear to be of equal importance. The administration of serotonin has been shown to result in the release of norepinephrine from cardiac sympathetic nerves, either through stimulation of serotonergic receptors on the nerve endings (Fozard and Mobarak, 1978) or by a tyramine-like action (Fillion et al., 1971). An inhibition of norepinephrine reuptake by sympathetic nerve endings also has been described (Borgen and Iversen, 1965; Horst and Jester, 1972). Thus, serotonin may increase cardiac contractility by enhancing the effect of cardiac norepinephrine release. Serotonin also has a direct positive inotropic effect on mammalian myocardium (Buccino et al., 1967; Sakai and Akima, 1979). This effect on contractility is not mediated by cyclic AMP (Benfey et al., 1974). There is evidence, at least in the mussel, for a direct effect on muscle calcium flux (Bloomquist and Curtis, 1972). It should be noted that cardiac serotonin, like cardiac norepinephrine, is not necessary for the maintenance of normal basal cardiac contractility (Buccino et al., 1967).

Another possible clinical significance for cardiac serotonin recently has emerged from the studies of Helke and co-workers (1978). These investigators have demonstrated an apparent role for peripheral serotonin in digitalis-induced cardiac arrhythmias. The infusion of serotonin into the cat resulted in a concomitant increase both in left ventricular serotonin content and in the susceptibility to arrhythmia induction during deslanoside infusion (Helke et al., 1978). Although treatment with drugs, such as serotonin antagonists or synthesis inhibitors, greatly increased the dose of deslanoside required to achieve toxicity, the depletion of brain serotonin alone had no ameliorating effect (Gillis et al., 1978).

Abnormalities in the availability of serotonin or tryptophan have been implicated in the pathogenesis of cardiomyopathy and muscular dystrophy. The rate-limiting step for serotonin production appears to be at the stage of tryptophan hydroxylation (Jequier et al., 1967). Under most physiological circumstances, the enzyme is unsaturated relative to its substrate tryptophan; thus, the availability of this amino acid to the enzyme appears to be a major determinant for the control of serotonin synthesis (Fernstrom and Wurtman, 1971). Dietary trypto-


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Modulating Effect of Regional Myocardial Performance on Local Myocardial Perfusion in the Dog

MARIO MARZILLI, SIDNEY GOLDSTEIN, HANI N. SABBAH, TENNYSON LEE, AND PAUL D. STEIN

SUMMARY We studied the effect of regional contractile performance on regional coronary blood flow and flow distribution in 10 dogs. The left anterior descending (LAD) coronary artery was cannulated and perfused. Maximal vasodilation was obtained with adenosine. Consequently, variations of LAD flow reflected changes of extravascular resistance. Lidocaine injected in the LAD caused a localized reduction of contractile performance as shown by the absence of systolic wall thickening. Global left ventricular performance and pressure were unchanged. Coronary extravascular resistance diminished and LAD flow increased from 4.8 ± 0.5 to 6.2 ± 0.6 ml/min per g (P < 0.001). Isoproterenol in the LAD augmented systolic wall thickening. Regional coronary flow diminished from 5.1 ± 0.5 to 3.3 ± 0.4 ml/min per g (P < 0.001), and the endocardial:epicardial ratio diminished from 1.08 ± 0.07 to 0.75 ± 0.07 (P < 0.01). These data indicate that myocardial contractility is a major component of extravascular coronary resistance and is a mechanical determinant of coronary blood flow and its transmural distribution. Circ Res 45: 634-641, 1979

THE interrelation of myocardial contractility and coronary blood flow has been studied extensively. It is well known that a reduction of regional perfusion results in immediate impairment of myocardial function (Goldstein and de Jong, 1974; Theroux et al., 1976; Heyndrickx et al., 1978). On the other hand, the effect of the state of myocardial contractility on coronary blood flow has received less attention. Systolic contraction has been shown to produce compression of the coronary vessels, thereby increasing their resistance to blood flow (Sabiston and Gregg, 1957; Kirk and Honig, 1964; Armour and Randall, 1971). This resistance to flow, which results from compression of the coronary vessels, is termed extravascular resistance, in contradistinction to the vascular resistance. The latter reflects changes of vessel diameter that result from coronary vasomotor tone.
Serotonin metabolism in the normal and failing hamster heart.
M J Sole, A Shum and G R Van Loon

Circ Res. 1979;45:629-634
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