J. Mol Cell Cardiol **17**, 1055–1063 (1985)

Digitoxin Therapy Partially Restores Cardiac Catecholamine and Brain Serotonin Metabolism in Congestive Heart Failure

Michael J. Sole*, Claude R. Benedict, Dirk H. G. Versteeg and E. Ronald de Kloet

Department of Medicine, Faculty of Medicine, University of Toronto, Toronto, Canada and The Rudolf Magnus Institute for Pharmacology, Utrecht, The Netherlands

(*Received 6 December 1984, accepted in revised form 7 March 1985*)

M. J. Sole, C. R. Benedict, D. H. G. Versteeg and E. R. de Kloet. Digitoxin Therapy Partially Restores Cardiac Catecholamine and Brain Serotonin Metabolism in Congestive Heart Failure. *Journal of Molecular and Cellular Cardiology* (1985) **17**, 1055–1063. The effect of therapeutic doses of digitalis in modifying neural activity has been the subject of considerable controversy. In earlier studies we reported an increase both in serotonergic activity in the posterior hypothalamus and pons-medulla and in cardiac sympathetic tone in the failing cardiomyopathic hamster. In this study we examine the effects of doses of digitoxin, known to be therapeutic for hamster heart failure, on monoamine neurotransmitter metabolism in the brain and heart during the cardiomyopathy. Both digitoxin and ASI–222, a polar amino-glycoside which does not cross the blood-brain barrier, given either acutely (6 mg/kg ip) or chronically (2 mg/kg/day ip for 10 days), normalized the failure-induced increase in serotonin turnover in the pons-medulla but had no effect on the changes in the posterior hypothalamus. Digitoxin therapy also reduced cardiac and adrenal sympathetic activity partially restoring cardiac catecholamine stores. In order to more clearly define the pathways involved we measured serotonin (μg/g protein) in 18 brain nuclei after 10 days of digitoxin or vehicle treatment. Heart failure was associated with an increase in serotonin in five nuclei: the mammillary; bodies, ventromedial, periventricular and paraventricular nuclei of the hypothalamus, and the centralis superior nucleus of the raphe. Digitoxin therapy completely normalized the changes in the centralis superior and ventromedialis nuclei; neither congestive heart failure nor digitoxin affected serotonin levels in other nuclei. We conclude that there is an increase in activity in specific brain serotonergic nuclei in congestive heart failure. Digitalis reduces cardiac sympathetic tone and restores the changes in two of these nuclei: the ventromedial and the centralis superior. These results suggest that at least two independent brain serotonin pathways are altered during heart failure and that perhaps a pathway between the centralis superior and ventromedialis nuclei participates in the integration of the increase in cardiac sympathetic tone found in heart failure.

Key Words: Digitalis; Hamster cardiomyopathy; Norepinephrine; Epinephrine; ASI-222; Nucleus centralis superior; Nucleus Ventromedialis; 5-hydroxytryptamine.

Introduction

Marked abnormalities in cardiovascular sympathetic and parasympathetic nerve traffic contribute to the inadequate hemodynamic performance of the failing heart. Such abnormalities may be attributed to disturbances not only in efferent autonomic nerves or their adjacent receptors but also in baroreceptor afferents and/or the processing of their signals within the central nervous system. In earlier studies [*30*, *32*] using a reproducible genetic paradigm of chronic congestive heart failure, the cardiomyopathic Syrian hamster, we showed a marked increase in cardiac sympathetic tone, which results in an increase in cardiac dopamine and a decrease in cardiac norepinephrine, was associated with myocardial decompensation. We also demonstrated an increase in serotonergic activity in the pons-medulla and posterior hypothalamus

* Address for reprint requests: Michael J. Sole, Room 12–222, Eaton North, Toronto General Hospital, Toronto, Ontario, Canada, M5G 1L7.

0022–2828/85/111055 + 09 $03.00/0

concomitant with these changes [*33*]. These data suggest that brain serotonin may be important for the integration of cardiovascular autonomic nerve traffic during heart failure.

Digitalis has been a mainstay of the therapy of congestive heart failure for two hundred years. The effect of digitalis in modifying central or peripheral neural activity has been the subject of some controversy (reviewed in *9*). Studies have documented the importance of both the parasympathetic and sympathetic innervation of the heart for the effects of the drug on electrophysiological parameters such as cardiac conduction, automaticity and refractory period. However, these data usually have been derived from non-failing preparations, often *in vitro* or under anesthesia, using toxic doses of the drug. There is very little information concerning the interaction of the autonomic nervous system with digitalis given in therapeutically appropriate doses as an inotropic agent for congestive heart failure. Digitoxin has been shown previously to ameliorate heart failure in the cardiomyopathic hamster [*2*]. Thus, in this study we examine the effects of therapeutic doses of digitoxin on both serotonin metabolism in the brain and catecholamine metabolism in the heart in hamster cardiomyopathy.

Methods

Male cardiomyopathic (Bio 53.58) and control (Bio RB) hamsters, 90–110 and 240–270 days of age, were used in these experiments. The younger cardiomyopathic animals exhibited early dystrophic changes in the heart but no stigmata of cardiac decompensation; the older animals manifested cardiac calcification and dilatation, hepatic engorgement and occasional ascites and subcutaneous edema. All hamsters were allowed at least 2 weeks to acclimatize to our animal facility following delivery from the breeder (Bio Research Institute, Cambridge, MA, USA). A 12 h light/dark cycle was maintained in the housing area. The animals were allowed water but deprived of food for 14 to 18 h prior to study; experiments were performed between 1000 to 1400 h.

Digitoxin (generously supplied by Eli Lilly and Co., Indianapolis, IN, USA) was dissolved in a vehicle of 25% alcohol and given in a dose of 2 mg/kg ip daily for 10 days in the chronic studies, or 6 mg/kg ip as a single dose in the acute study. ASI–222 (3 β-0-(4 amino-4,6 dideoxy-β-D-galactopyranosyl) digitoxigenin; Ash-Stevens, Detroit, MI, USA) was made up similarly. Three hours after the last dose of drug (or vehicle alone) hamsters were decapitated. In the studies examining the major brain areas, the brain was cleaned and dissected on an ice-cooled glass plate into five regions [*31*]: pons-medulla, mid brain, posterior and anterior hypothalamus and cerebellum. The full thoracic cord was also taken. The tissues were then immediately frozen on dry ice, weighed and processed for analysis. The preparation and assay of tissues for serotonin and 5HIAA in the chronic studies was performed as described by Sole *et al.* [*33*] using the spectrofluorometric method of Curzon and Green [*7*]. In the acute studies, serotonin turnover was estimated by the accumulation of 5HTP following inhibition of aromatic L-amino acid decarboxylase with m-hydroxybenzylhydrazine (Aldridge Chemical Co., Milwaukee, WI, USA; 100 mg/kg, ip). Accumulation of 5HTP was linear for at least 30 min in the brain regions examined. The preparation of tissues and the analysis of 5HTP by high-performance liquid chromatography with electrochemical detection has been described previously by us ([*26*]; System 1).

In the nuclear studies, the brains were frozen whole on dry ice and stored at $-70°C$ for a maximum of 7 days before sectioning and nuclear punching. Brains were cut into 300 μm sections in a cryostat at $-10°C$. Nuclei were punched with hollow needles using criteria modified from Palkovits [*20*]. Eighteen individual brain regions were taken from each hamster and assayed separately (Fig. 4). The centralis superior and median raphe nuclei were taken together; this region will be called centralis superior in this study. The individual nuclei were prepared and assayed for serotonin exactly as described by Hussain and Sole [*14*]. The catecholamines, dopamine, norepinephrine and epinephrine, were measured in hamster hearts in some of these studies. The preparation of the hearts and the radioenzymatic assay of the catecholamines is described in detail by Sole *et al.* [*30*].

Data are given as mean ± standard error of the mean. At least seven animals constituted each group. Significance among the groups was determined by one way analysis of variance with comparison among the individual means according to the Q-test [28].

Results

Failing hamsters exhibited an increase in cardiac dopamine and a reduction in cardiac norepinephrine stores, as we reported previously (Fig. 1). In addition, we noted an increase in cardiac epinephrine concentration in heart failure. The administration of a 'therapeutic' regimen of digitoxin resulted in a partial normalization of cardiac catecholamine concentration; that is, cardiac norepinephrine increased while cardiac dopamine and epinephrine fell.

Measurement of serotonin and its major metabolite 5HIAA in brain regions confirmed our earlier observation that both indoles were increased in the pons-medulla and posterior hypothalamus in heart failure (Table 1). Cardiomyopathic hamsters in early stages of their disease exhibited no differences from controls. The administration of digitoxin completely restored the alterations in the pons-medulla to control values but had no effect on the

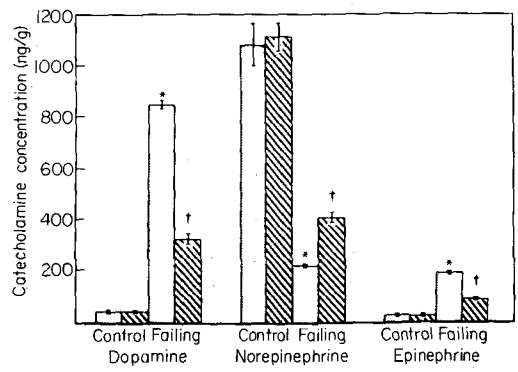

FIGURE 1. Effect of digitoxin on cardiac (ventricles only) catecholamines in control and failing hamsters. Digitoxin was administered in a dose of 2 mg/kg ip as a single dose daily for 10 days. (□: Vehicle treated; ▨: Digitoxin treated.) * $P < 0.05$ v. control hamsters. † $P < 0.05$ v. both control hamsters and vehicle treated myopathic hamsters.

changes exhibited by the posterior hypothalamus (Table 1).

In order to determine whether digitoxin acted either directly in the brain to elicit this effect or indirectly via a more peripheral site, we examined brain 5HTP accumulation, a measure of serotonin synthesis, following the administration of ASI–222, a polar amino glycoside which does not cross the blood-brain barrier [18]. 5HTP accumulation was increased during heart failure only in the

TABLE 1. Effect of Digitoxin (2 mg/kg/day ip × 10 days) on Brain Serotonin Metabolism in Control and Failing Hamsters

	Control		Failing	
	Vehicle	Digitoxin	Vehicle	Digitoxin
5HT (μg/g)				
Midbrain	1.89 ± 0.04	1.82 ± 0.04	1.89 ± 0.04	1.88 ± 0.03
Pons-medulla	1.57 ± 0.04	1.61 ± 0.02	1.97 ± 0.03[a]	1.67 ± 0.04
Anterior hypothalamus	2.22 ± 0.05	2.34 ± 0.05	2.32 ± 0.05	2.33 ± 0.05
Posterior hypothalamus	2.53 ± 0.05	2.58 ± 0.09	3.19 ± 0.05[b]	3.19 ± 0.04[b]
Thoracic cord	1.89 ± 0.03	1.91 ± 0.03	1.95 ± 0.03	1.95 ± 0.04
5HIAA (μg/g)				
Midbrain	1.09 ± 0.02	1.03 ± 0.03	1.15 ± 0.02	1.11 ± 0.01
Pons-medulla	0.93 ± 0.04	0.99 ± 0.07	1.32 ± 0.03[a]	1.12 ± 0.06
Anterior hypothalamus	1.19 ± 0.01	1.15 ± 0.04	1.20 ± 0.04	1.17 ± 0.03
Posterior hypothalamus	1.21 ± 0.03	1.24 ± 0.03	1.54 ± 0.03[b]	1.50 ± 0.03[b]
Thoracic cord	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.02	0.37 ± 0.01

[a] $P < 0.005$ v. digitalized cohort or vehicle treated control.
[b] $P < 0.05$ v. matched controls.
5HT = Serotonin; 5HIAA = 5-hydroxyindoleacetic acid.

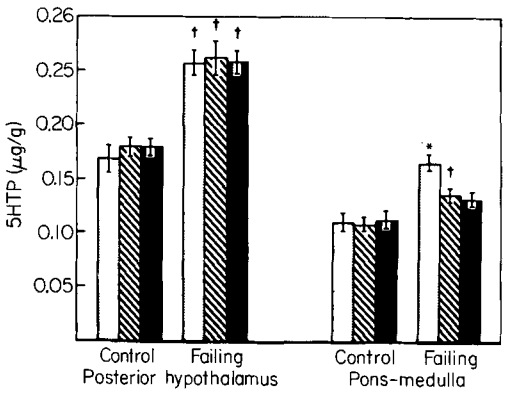

FIGURE 2. The effect of a single dose (6 mg/kg ip) of digitoxin or ASI–222 on brain serotonin synthesis, as measured by 5HTP accumulation, in control and failing hamsters. 5HTP was assayed 30 min following the inhibition of aromatic L-amino acid decarboxylase. (□: Vehicle treated; □: Digitoxin treated; ■: ASI–222 treated.) $*P < 0.05$ v. both control hamsters and hamsters treated with digitoxin or ASI–222. $\dagger P < 0.05$ v. control hamsters.

TABLE 2. Serotonin content (μg/g protein) in brain nuclei of control hamsters

Hypothalamic nuclei	
Supraopticus (so)	10.81 ± 0.71
Periventricularis (npe)	8.30 ± 0.29
Paraventricularis (npv)	16.28 ± 1.18
Arcuatus (na)	31.23 ± 1.25
Ventromedialis (nvm)	9.64 ± 0.31
Dorsomedialis (ndm)	15.03 ± 0.75
Posterior hypothalamicus (nhp)	23.12 ± 1.09
Median eminence (me)	12.25 ± 0.66
Mammillary bodies (mb)	7.44 ± 0.26
Raphe nuclei	
Raphe dorsalis (rd)	23.61 ± 1.15
Centralis superior (ncs)	16.88 ± 0.59
Raphe magnus (rm)	15.68 ± 1.50
Raphe pallidus (rp)	8.74 ± 0.47
Pons-medulla nuclei	
Cuneatus (ncu)	5.72 ± 0.35
Locus ceruleus (lc)	10.82 ± 0.64
Area postrema (ap)	16.51 ± 0.78
Tractus solitarius (nts)	9.93 ± 0.63
Commisuralis (nco)	17.69 ± 0.35

pons-medulla and posterior hypothalamus (Fig. 2) supporting our assertion in the previous experiments that the increase in both serotonin and 5HIAA reflected an increase in serotonin turnover. A single dose of either digitoxin or ASI–222 was able to restore serotonergic activity in the pons-medulla towards normal; again no effect was noted in the posterior hypothalamus (Fig. 2).

As brain regions such as the pons-medulla or posterior hypothalamus are comprised of a plethora of serotonin pools, many of which are certain not to participate in neural effects important in congestive heart failure, we next proceeded to develop a primitive central serotonin map of heart failure by examining specific serotonergic brain nuclei. The levels of serotonin in various brain nuclei of control hamsters are recorded in Table 2. Serotonin was found in each of the nuclei with the highest values recorded in the nuclei arcuatus and raphe dorsalis. We found no difference in the serotonin concentration of any of these nuclei between young adult myopathic and control hamsters. However, with the advent of congestive heart failure, serotonin increased in five specific nuclear regions (Fig. 3): the mammillary bodies, ventromedial, periventricular and paraventricular nuclei of the hypothalamus and the centralis superior nucleus (a raphe nucleus). The ventromedial and centralis superior nuclei exhibited close to 100% increase in their serotonin levels (Table 3, Fig. 3). Treatment of failing hamsters with digitoxin completely restored the changes in the nucleus centralis superior and the nucleus ventromedialis to normal (Table 3, Fig. 4): The serotonin concentrations of the other brain nuclei (including the periventricular and paraventricular nuclei and the mammillary bodies) were not affected.

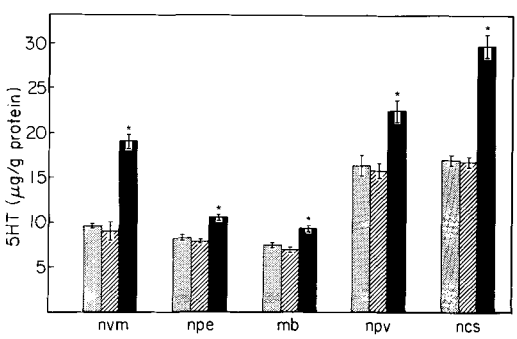

FIGURE 3. Serotonin (5HT) concentration in brain nuclei of control and cardiomyopathic hamsters. The key to the abbreviation for the various nuclei may be found in Table 2. (□: control; □: myopathic young; ■: myopathic failing.) $*P < 0.01$ v. both control and young cardiomyopathic hamsters.

TABLE 3. The effect of chronic treatment with digitoxin on serotonin levels (μg/g protein) in specific brain nuclei of control and failing hamsters

	Vehicle		Digitoxin	
Nuclei	Control	Failing	Control	Failing
npe	8.30 ± 0.31	10.51 ± 0.37[a]	8.11 ± 0.40	9.72 ± 0.31
npv	16.28 ± 1.18	22.35 ± 1.21[a]	15.69 ± 0.53	22.01 ± 1.03
nvm	9.64 ± 0.31	19.07 ± 0.66[a]	9.18 ± 0.51	10.51 ± 0.70[b]
mb	7.44 ± 0.26	9.25 ± 0.36[a]	7.56 ± 0.66	10.81 ± 0.96
ncs	16.88 ± 0.59	29.58 ± 1.35[a]	16.49 ± 0.31	16.51 ± 0.80[b]

[a] $P < 0.01$ v. control cohort.
[b] $P < 0.01$ v. vehicle treated failing animals.
Abbreviations used are the same as in Table 2.

Discussion

Heart failure in hamster cardiomyopathy is associated with a depletion of cardiac norepinephrine stores and an increase in the levels of cardiac dopamine [*30*]. This abnormal distribution of cardiac catecholamines appears to be secondary to a marked increase in the sympathetic tone of the decompensating heart for it is readily abolished by peripheral ganglionic blockade and can be mimicked in normal animals by prolonged stress [*30*, *32*]. In this study we now show that an increased level of epinephrine is also present in failing hamster ventricles. Epinephrine is not synthesized in the heart, but in the adrenal, and is taken up into cardiac sympathetic nerve endings as it passes through the coronary circulation. Thus, the increase in myocardial epinephrine

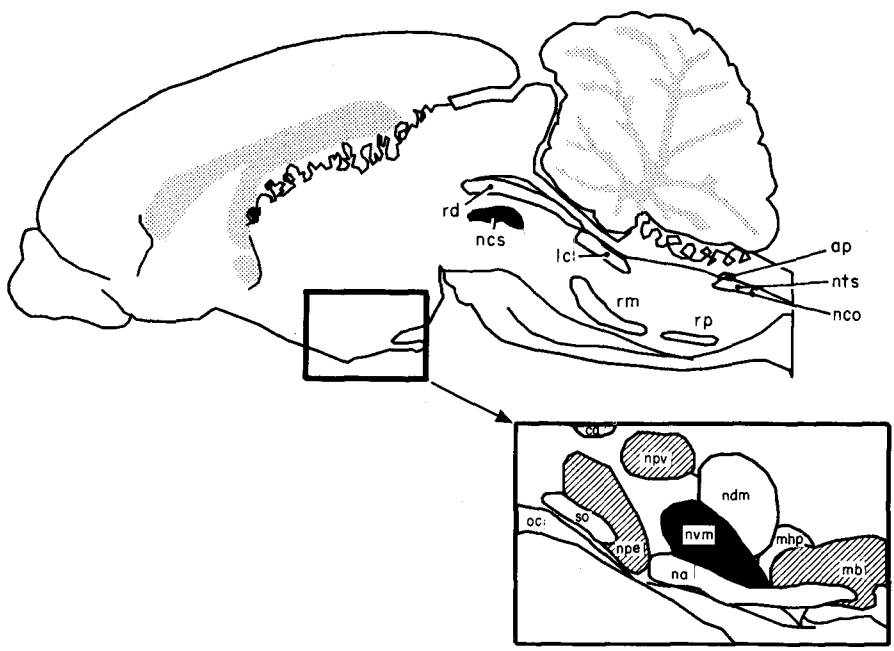

FIGURE 4. Saggital section of hamster brain illustrating the location of the various nuclei examined. The black nuclei represent regions in which serotonin was increased in heart failure and normalized by digitoxin. The hatched nuclei represent those affected by heart failure but not by digitoxin. Nuclei, shown in outline only, were unaffected by either failure or treatment. For the key to the abbreviations see Table 2. The ncu and me are not shown.

in hamster heart failure reflects an increase in sympathoadrenal tone associated with the failing state.

Digitoxin administered in a dose of 2 mg/kg/day has been shown to ameliorate hamster heart failure; hamsters could be maintained on this regimen for several months without toxicity [*2*]. Digitoxin decreased both cardiac epinephrine and dopamine and partially restored cardiac norepinephrine. This tendency to normalize myocardial catecholamines was similar to the effect of ganglionic blockade [*30*]. When digitalis is given in heart failure indirect evidence, such as the reduction of forearm vascular resistance, has suggested that there is a reduction in the previously elevated sympathetic tone [*8, 9, 17*]. Our data provide evidence that therapeutic doses of digitalis can reduce cardiac (and adrenal) sympathetic activity in heart failure. These findings cannot be attributed to the ability of toxic doses of digitalis glycosides to decrease uptake or stimulate release of norepinephrine in tissues *in vitro* [*9, 24, 25*].

A reduction in sympathetic tone during heart failure may account for some of the beneficial electrophysiological effects of digitalis. The sympathetic innervation of the failing heart is heterogeneous [*29*]. In this setting, an increase in sympathetic tone would be expected to predispose the heart to significant, perhaps lethal, ventricular arrhythmias. Although the clinical indications for digitalis as an antiarrhythmic drug are relatively restricted to supraventricular arrhythmias, the drug can be clinically useful for ventricular arrhythmias in heart failure. Such a therapeutic effect may be a manifestation of this ability of digitalis to reduce cardiac sympathetic tone. As a further consideration, a reduction in sympathetic tone, in human correlates of hamster cardiomyopathy theoretically may mask the positive inotropic effect of chronic digitalis administration and, thus, may contribute to the controversy of whether or not chronic digitalis therapy is of hemodynamic benefit to patients in heart failure and in sinus rhythm [*11*]. This suggestion is supported by a study by Horowitz *et al.* [*13*] in which an exercise-induced increase in sympathetic tone, in dogs, appeared to obviate any positive inotropic effects of digitalis.

The reduction of sympathetic tone by digitalis may merely reflect an autonomic response to the stimulation of myocardial contractility by the drug. However, there is considerable evidence to suggest that direct neural affects of digitalis play a major role. Cardiovascular autonomic tone is regulated by a stream of afferent neural signals which are integrated in the cardiovascular centers of the central nervous system. The increase in sympathetic activity seen in heart failure at least partially, reflects a breakdown in this control system. A major component of this dysfunction appears to be a decrease in the sensitivity of baroreceptors which mediate the generation of cardioinhibitory afferent nerve traffic [*8*]. Recent studies have indicated that digitalis can increase the sensitivity of these receptors and, thus, may partially normalize the buffering of cardiovascular autonomic neurogenic drive [*8, 22, 35*].

The identification and localization of brain neurotransmitters that participate in the integration of cardiac autonomic nerve traffic during heart failure and their response to therapy by digitalis should be of importance in furthering our understanding of the pathophysiology of congestive heart failure and the therapeutic actions of this venerable class of drugs. Brain serotonin appears to be important in the regulation of cardiovascular function (reviewed in [*12, 15*]). However, elucidation of its precise role has been difficult. In the rat and rabbit, brain serotonergic activity appears to parallel sympathetic tone while the opposite appears true in the dog and cat. However, it should be noted that some data which conflicts with these conclusions also exist in the literature. Serotonergic neurons in different brain regions may subserve opposing functions; such differences may be obscured by the more widespread effects of centrally or peripherally administered pharmacological agents. A cardiovascular response following the manipulation of central serotonin suggests but does not actually demonstrate that a given serotonergic pathway participates in the central regulation of this response under physiological conditions in the normal or diseased animal. An examination of serotonin metabolism and turnover in specific brain regions under these conditions can provide such information. In an earlier study we demonstrated an increase

in both serotonin and its major metabolite 5HIAA, hence serotonergic activity, in both the pons-medulla and the posterior hypothalamus during hamster heart failure [*33*]. No changes were seen in young non-failing animals. The current study confirms these data using two independent assessments of serotonin turnover. In addition, we now show that digitoxin given even as a single dose, can restore serotonergic activity in the pons-medulla while no effect is seen in the posterior hypothalamus. ASI–222, a polar glycoside which does not cross the blood-brain barrier [*18*], appears to be equally effective. This latter experiment suggests that the central restorative action of digitoxin is mediated through some peripheral afferent mechanism such as a sensitization of cardiopulmonary baroreceptors. It is also possible that digitoxin and ASI–222 both act at a central neural locus in which the blood-brain barrier is defective or absent. Indeed, the emetic affect of toxic doses of digitalis appears to represent stimulation of such an 'unprotected' brain region, the area postrema [*5, 9*].

The dissociation between the serotonergic activity in the pons-medulla and posterior hypothalamus suggests that heart failure can induce alterations in at least two independent serotonergic pathways. Brain regions such as the pons-medulla and posterior hypothalamus are comprised of a plethora of serotonin pools; thus events occurring in specific functional areas may be obscured by changes, or the lack of changes, in others. In order to more clearly define the pathways involved and the role of the blood-brain barrier we did a further study using nuclear punch techniques and a sensitive radioenzymatic serotonin assay. The serotonin contents of the nuclear regions we examined were remarkably similar to those found in the rat [*21, 23*]. No differences in serotonin content between control and young myopathic hamsters were found in any of the nuclei examined. However, with the advent of congestive heart failure, serotonin was increased in the mammillary bodies, the ventromedial, periventricular and paraventricular nuclei of the hypothalamus, and the centralis superior nucleus in the raphe; digitalis therapy restored the increases in the ventromedial nucleus and in the centralis superior nucleus to normal.

Serotonergic axons of the nucleus centralis superior project rostrally via the medial subcortical pathway; a portion of this pathway diverges ventromedially to travel in the median forebrain bundle to supply the hypothalamus [*1, 19*]. The nucleus centralis superior also appears to have some caudally projected fibers [*10*]. Electrical stimulation of the nucleus centralis superior (which is confluent with the nucleus medianis or region B8; 21) in the rat produces a stimulus dependent increase in blood pressure [*16, 27*]. This response is abolished if brain serotonin is first depleted by p-chlorophenylalanine administration [*16, 27*]. Conversely, we have shown in experiments in the rat that serotonergic activity in the nucleus centralis superior is inhibited by the stimulation of cardiac vagal afferents [*34*].

Little information exists concerning the possible role of the ventromedial nucleus in cardiovascular regulation. The serotonin content of this nucleus has been shown to increase with immobilization stress [*6*]. Electrical stimulation of, or the injection of serotonin into, this general region of the hypothalamus leads to sympathetic excitation and an increase in blood pressure [*4*], similar to the response to stimulation of the nucleus centralis superior. Injection of ouabain directly into the ventromedial hypothalamus is reported to produce a bradycardia which can be abolished by vagotomy [*3*]; however, such high local concentrations of digitalis would not be achieved in our experiments. Stimulation of cardiac vagal afferents during myocardial infarction has no effect on serotonergic activity in the ventromedial nucleus [*34*].

These data suggest that the centralis superior and perhaps ventromedial nuclei participate in the integration of information from baroreceptor afferents. However, it should be noted that both of these nuclei also appear to be important in neuroendocrine regulation (reviewed in [*1*]); thus, alterations in their serotonergic activity may also represent their participation in the control of some of the hormonal changes associated with congestive heart failure.

Finally, it is also worthwhile to note that we found no changes in serotonin in the area postrema, or in the nucleus tractus solitarii,

including the nucleus commisuralis, the primary termini for the cardiac vagus (and the cardiovascular reflex arc). Cardiac vagal stimulation by myocardial infarction also failed to alter serotonin in these regions [*34*]. Thus, the serotonergic innervation of these brain regions do not appear to participate in the cardiopulmonary baroreflex response.

In conclusion, congestive heart failure, at least as exhibited by the cardiomyopathic hamster, is associated with an increase in serotonergic activity in the centralis superior nucleus of the raphe, the mammillary bodies, and the periventricular, paraventricular and ventromedial nuclei of the hypothalamus. The administration of therapeutic doses of digitoxin in heart failure restores the alterations in two nuclei, the nucleus centralis superior and the nucleus ventromedialis to that found in normal hamsters; the other nuclei were not affected. In addition, we have shown that digitoxin given in therapeutic doses for hamster heart failure reduces cardiac (and adrenal) sympathetic activity.

Some or many of the changes we observed in brain serotonin probably underlie effects distinct from those which directly affect or modulate cardiovascular autonomic activity. However, our observations suggest that heart failure is associated with alterations in the activity of at least two independent brain serotonin pathways and that perhaps one of these pathways, possibly between the centralis superior and ventromedialis nuclei, participates in the modulation or integration of the increase in cardiac sympathetic tone found in heart failure.

Acknowledgements

This study was supported by the U.S. Public Health Service and the Ontario Heart Foundation. Dr Sole is a Research Associate of the Ontario Heart Foundation.

References

1 Azmitia, E. C. The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In: *Handbook of Psychopharmacology*, L. L. Iversen, S. D. Iversen, S. H. Snyder (Eds), pp. 233–314. New York: Plenum. **9** (1978).

2 Bajusz, E., Homburger, F., Baker, J. R., Bogdonoff, P Dissociation of factors influencing myocardial degeneration and generalized cardiocirculatory failure. Ann NY Acad Sci **156**, 397–420 (1969).

3 Basu Ray, B. N., Booker, W. M., Dutta, S. M., Pradhan, S. N. Effects of microinjection of ouabain into the hypothalamus in cats. Br J Pharmacol **45**, 197–206 (1972).

4 Calaresu, F. R., Thomas, M. R. Electrophysiological connections in the brainstem involved in cardiovascular regulation. Brain Res **87**, 335–338 (1980).

5 Chai, C. Y., Hsu, P. L., Wang, S. C. Central locus of emetic action of digitalis substances in cats. Neuropharmacology **12**, 1187–1193 (1973).

6 Culman, J., Kvetnansky, R., Torda, T., Murgas, K. Serotonin concentration in individual hypothalamic nuclei of rats exposed to acute immobilization stress. Neuroscience **5**, 1503–1506 (1980).

7 Curzon, G., Green, A. R. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Br J Pharmacol **39**, 653–655 (1970).

8 Ferguson, D. W., Abboud, F. M., Mark, A. L. Selective impairment of baroreflex-mediated vasoconstrictor responses in patients with ventricular dysfunction. Circulation **69**, 451–460 (1984).

9 Gillis, R. A., Quest, J. A. The role of the nervous system; in the cardiovascular effects of digitalis. Pharmacol Rev **31**, 19–97 (1980).

10 Halaris, A. E., Jones, B. E., Moore, R. Y. Axonal transport in serotonin neurons of the mid brain raphe. Brain Res **107**, 555–574 (1976).

11 Hamer, J. The paradox of the lack of the efficacy of digitalis in congestive heart failure with sinus rhythm. Br J Clin Pharmacol **8**, 109–113 (1979).

12 Head, G. A., Korner, P. I. Cardiovascular functions of brain serotonergic neurons in the rabbit as analysed from the acute and chronic effects of 5,6-dihydroxytryptamine. J Cardiovasc Pharmacol **4**, 398–408 (1982).

13 Horowitz, L. D., Atkins, J. M., Seite, M. Effect of digitalis on left ventricular function in exercising dogs. Circ Res **41**, 744–749 (1977).

14 Hussain, M. N., Sole, M. J. A simple, specific, radioenzymatic assay for picrogram quantities of serotonin or acetylserotonin in biological fluids and tissues. Annal Biochem **111**, 105–110 (1981).

15 Kuhn, D. M., Wolf, Wm. A., Lovenberg, W. Review of the role of the central serotonergic neuronal system in blood pressure regulation. Hypertension **2**, 243–255 (1980).

16 Kuhn, D. M., Wolf, W. A., Lovenberg, W. Pressor effects of electrical stimulation of the dorsal and median raphe nuclei in anesthetized rats. J Pharmacol Exp Ther **214**, 403–409 (1980).

17 Mason, D. T., Braunwald, E. Studies on digitalis X. Effects of ouabain on forearm vascular resistance and venous tone in normal subjects and in patients in heart failure. J Clin Invest **43**, 532–543 (1964).
18 Mudge, G. H., Lloyd, B. L., Greenblatt, D. J., Smith, T. W. Inotropic and toxic affects of a polar cardiac glycoside derivative in the dog. Circ Res **43**, 847–854 (1978).
19 Palkovits, M. Topography of chemically identified neurons in the central nervous system: a review. Acta Morphologica Acad Sci Hung **26**, 211–290 (1978).
20 Palkovits, M. Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res **59**, 449–450 (1973).
21 Palkovits, M., Brownstein, M., Saavedra, J. M. Serotonin content of the brain stem nuclei in the rat. Brain Res **80**, 237–249 (1974).
22 Quest, J. A., Gillis, R. A. Effect of digitalis on carotid sinus baroreceptor activity. Circ Res **35**, 247–255 (1974).
23 Saavedra, J. M., Palkovits, M., Brownstein, M. J., Axelrod, J. Serotonin distribution in the nuclei of the rat hypothalamus and preoptic region. Brain Res **77**, 157–165 (1974).
24 Seifen, E. Evidence for participation of catecholamines in cardiac action of ouabain: release of catecholamines. Eur J Pharmacol **26**, 115–118 (1974).
25 Sharma, V. K., Banerjee, S. P. Inhibition of ^{3}H-norepinephrine uptake in peripheral organs of some mammalian species by ouabain. Eur J Pharmacol **41**, 417–429 (1977).
26 Shum, A., Sole, M. J., Van Loon, G. R. Simultaneous measurement of 5-hydroxytryptophan and L-dihydroxyphenylalanine by high performance liquid chromatography with electrochemical detection: measurement of serotonin and catecholamine turnover in discrete brain regions. J Chromatol **228**, 123–130 (1982).
27 Smits, J. F. M., Van Essen, H., Struyker-Boudier, H. A. J. Serotonin-mediated cardiovascular responses to electrical stimulation of the raphe nuclei in the rat. Life Sci **23**, 173–178 (1978).
28 Snedecor, G. W., Cochran, W. G. *Statistical Methods*. Ames, Iowa: Iowa State University Press (1972).
29 Sole, M. J. Alterations in sympathetic and parasympathetic neurotransmitter activity. In: *Congestive Heart Failure*. E. Braunwald, M. B. Mock, J. T. Watson (Eds), pp. 101–113, New York: Grune and Stratton (1982).
30 Sole, M. J., Helke, C. J., Jacobowitz, D. M. Increased dopamine in the failing hamster heart: transvesicular transport of dopamine limits the rate of norepinephrine synthesis. Am J Cardiol **49**, 1682–1690 (1982).
31 Sole, M. J., Hussain, M. N., Lixfeld, W. Activation of brain catecholaminergic neurons by cardiac vagal afferents during acute myocardial ischemia in the rat. Circ Res **47**, 166–175 (1980).
32 Sole, M. J., Lo, C-M., Laird, C. W., Sonnenblick, W. H., Wurtman, R. J. Norepinephrine turnover in the heart and spleen of the cardiomyopathic Syrian hamster. Circ Res **27**, 855–862 (1975).
33 Sole, M. J., Shum, A., Van Loon, G. R. Alterations in brain serotonin during congestive heart failure in the cardiomyopathic Syrian hamster. Cardiovasc Res **12**, 373–375 (1978).
34 Sole, M. J., Versteeg, D. H. G., de Kloet, E. R., Hussain, M. N., Lixfeld, W. The identification of specific serotonergic nuclei inhibited by cardiac vagal afferents during acute myocardial ischemia in the rat. Brain Res **265**, 55–61 (1983).
35 Thames, M. D., Miller, B. D., Abboud, F. M. Sensitization of vagal cardiopulmonary baroreflex by chronic digoxin. Am J Physiol **243**, H815–H818 (1982).