# Serotonin is localized in endothelial cells of coronary arteries and released during hypoxia: A possible new mechanism for hypoxia-induced vasodilatation of the rat heart [1]

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*Summary.* In this report we demonstrate the immunocytochemical localization of serotonin in endothelial cells of rat coronary vessels and a significant increase in the release of serotonin into the perfusate of Langendorff rat heart preparations during hypoxia. It is suggested that serotonin, localized in endothelial cells, is released during hypoxia and could provide part of a pathophysiological mechanism for vasodilatation to protect the heart from damage due to hypoxia.
*Key words.* Heart; hypoxia; serotonin; endothelial cells.

In 1980 Furchgott and Zawadzki [2] reported that the relaxation of arterial smooth muscle by acetylcholine (ACh) required the presence of endothelial cells. Since this time many other agents have been shown to act via receptors on endothelial cells to release an endothelium-derived relaxing factor (EDRF) [3]. One problem in establishing a physiological role for such a mechanism is to identify the source of the agents that produce the vasodilator responses via endothelial cells. Since periarterial nerves are confined to the adventitial-medial border of blood vessels, it is unlikely that release of ACh from nerves provides the answer, as it would require diffusion of the agent through the medial muscle coat (where it usually initiates vasoconstriction) before it could act on the endothelial cells to release EDRF. The first evidence for a source of ACh other than in periarterial nerves was obtained in this laboratory when the synthetic enzyme, choline acetyltransferase, was localized at the ultrastructural level in vascular endothelial cells in rat brain [4]. An endothelium-dependent relaxation to serotonin (5-hydroxytryptamine, 5-HT) has been reported in isolated coronary arteries [5] and in rabbit whole heart preparations [6] and this response has been discussed in the context of the large amounts of 5-HT that can be released from platelets during aggregation [5, 7]. However, some blood vessels, including coronary arteries, have been shown to produce an endothelium-dependent vasodilatation in response to hypoxia [8, 9] in preparations perfused with physiological saline, thus excluding release from platelets as a source of 5-HT or other vasoactive substances. In the present investigation we have examined the possibility that 5-HT may be stored in endothelial cells of coronary vessels and be released during hypoxia of the heart to contribute to the resulting vasodilatation.

*Methods.* Eight adult Wistar rats were anaesthetized with ether and perfused via the left ventricle with 3% paraformaldehyde, 0.1% glutaraldehyde and 15% saturated picric acid in 0.1 M phosphate buffer (pH 7.3). Segments of coronary vessels were removed and placed in glutaraldehyde-free fixative overnight at 4 °C. Sections (40 µm) were cut on a Vibratome and were rinsed for 24 h in several changes of phosphate-buffered saline. Sections were incubated for 48 h at 4 °C with antisera to 5-HT raised in rabbits (Immuno Nuclear Corporation, No. 4342T), at dilutions of 1:500 and 1:1000. Following treatment with 3,3'-diaminobenzidine and hydrogen peroxide, sections were transferred to 0.5% osmium tetroxide for 30 min, dehydrated through an ethanol series and embedded in Araldite. Control experiments were carried out with antiserum preincubated for 12 h with excess antigen (100 µm 5-HT creatinine sulphate per ml diluted antiserum). No reaction product was observed in control sections.

Fourteen adult Wistar rats were injected with heparin (2500 units, i.p.) before being killed by a blow to the head and exsanguination. The heart was removed and cannulated via the aorta for constant flow perfusion at a starting perfusion pressure of 50–60 mm Hg. This procedure has been described in detail previously [10]. The heart was paced at 4 Hz with electrical pulses of 5 ms duration and supramaximal voltage. The hearts were perfused for 20 min before a 200-µl bolus of $10^{-4}$ M ergotamine was given. This raised the perfusion pressure by an average of 50 mm Hg. Hypoxia was induced by switching from Krebs Henseleit solution equilibrated with 95% $O_2$, 5% $CO_2$ to one equilibrated with 95% $N_2$, 5% $CO_2$ via a second perfusion system with a rate-matched pump. After 1 min, the hypoxic perfusion was ended by switching back to the original oxygenated solution. Control experiments were carried out using exactly the same procedure, except that normoxic Krebs solution replaced the hypoxic Krebs solution in the second perfusion system. Throughout the experiments, perfusion pressure and left ventricular pressure were monitored. Fractions (12 s dura-

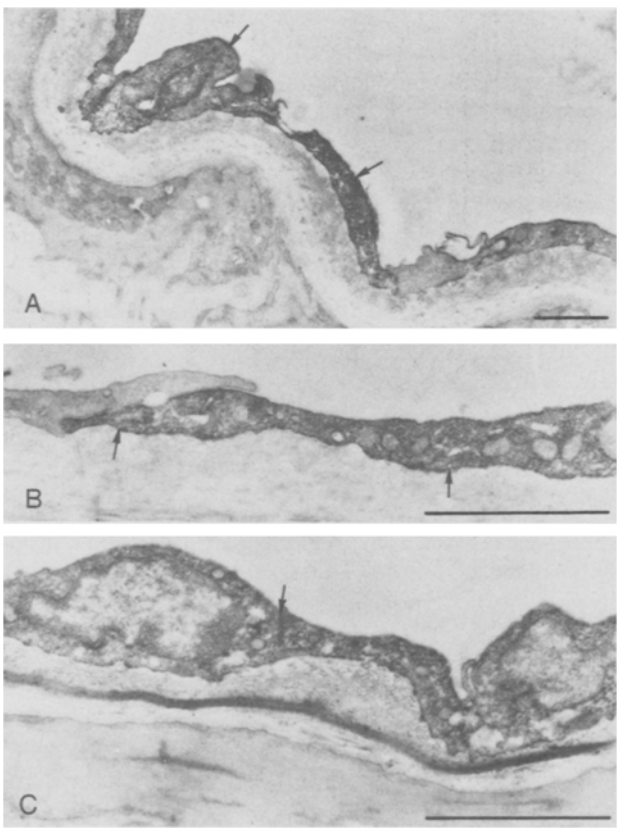


Figure 1. Ultrastructural localization of 5-HT immunoreactivity in left (A and B) and right (C) rat coronary arteries. Calibration bars = 1 µm. Arrows indicate 5-HT-immunoreactive endothelial cells in A, B and C. Note that the adjacent cells are not labeled.

tion) of cardiac effluent were collected in ice-cold polypropylene tubes and kept on ice. Levels of 5-HT and its breakdown product, 5-hydroxyindoleacetic acid (5-HIAA) were measured in the perfusate using high performance liquid chromatography with electrochemical detection by the method of Reinhard et al.[11]. Quantitation was achieved using a glassy carbon electrode set at a potential of + 0.72 V. Levels of 5-HT and 5-HIAA were measured by comparing peak heights and retention times with standards. Results were calculated as indoles (5-HT + 5-HIAA) per fraction per g wet weight heart. Statistical comparison between equivalent fractions in hypoxic and control experiments was achieved using Student's one-tailed t-test.

*Results.* Examination of the ultrathin sections with the electron microscope consistently revealed the presence of 5-HT immunoreactivity in approximately 50% of the endothelial cells of coronary vessels (fig. 1, A). Organelles observed in the endothelial cells included the nucleus, Golgi apparatus, mitochondria and rough endoplasmic reticulum (fig. 1, B, C). A large number of caveolae or vesicles were seen associated with both the adluminal and the abluminal surfaces of the endothelial cells (fig. 1, B).

In the Langendorff heart preparations, switching to hypoxic Krebs solution rapidly produced vasodilatation as indicated by a decrease in the perfusion pressure followed by a recovery phase on returning to oxygenated Krebs solution (fig. 2, A). Analysis of the cardiac effluent for 5-HT and 5-HIAA revealed a significant increase in the levels released which occurred rapidly after the onset of hypoxia, reaching a peak at the time of maximal vasodilatation (fig. 2, A). No such increase was observed in the control experiments (fig. 2, B), indicating that the release did not occur as a consequence of mechanical changes in pressure caused by switching perfusion systems.

*Discussion.* The results of the present investigation provide evidence for the presence of 5-HT in endothelial cells of rat coronary arteries and demonstrate that 5-HT is released during hypoxic vasodilatation of the heart. Since 5-HT has not been demonstrated in nerves in the heart in vivo, it appears likely that the source of 5-HT released was the endothelial cells. It was previously shown that choline acetyltransferase was localized in endothelial cells of rat brain vessels and suggested that choline could be taken up from the circulation and synthesized into ACh by the enzyme stored in the endothelial cells[4]. From the results reported here, it is not possible to conclude whether 5-HT is synthesized in the endothelial cells or taken up from the circulation and stored. Endothelial cells from bovine aorta have been shown to take up and metabolize 5-HT in culture[12].

Studies have been carried out on the response of coronary arteries to 5-HT and to aggregating platelets[5,7]. From these experiments it has been suggested that the endothelial cells may protect coronary vessels from a direct vasoconstrictor effect on the vascular smooth muscle by 5-HT and other substances released from platelets during aggregation. In addition, it has been speculated that coronary vasospasm may be initiated at sites where the endothelium has been damaged[5,7]. The present study raises the possibility that the endothelium may also make a more active contribution to the regulation of blood vessels by the release of its own stores of 5-HT in response to stimuli such as hypoxia.

In addition to 5-HT, ACh, ATP, substance P, bradykinin and thrombin have all been shown to produce endothelium-dependent relaxations of coronary vessels[3]. It has already been hypothesized that there may be interactions between peptides and non-peptides released from perivascular nerves and from endothelial cells[13]. Work is in progress to investigate whether these agents are also stored in endothelial cells and released during hypoxia. ATP has been shown to be released during hypoxia of the guinea pig heart[14]. Preliminary immunocytochemical studies have localized choline acetyltransferase and substance P in endothelial cells of coronary vessels and, although variable, there have been indications that levels of ACh and substance P in the cardiac effluent are increased in hypoxia (unpublished observations). It is therefore possible that some agents which produce EDRF-mediated vasodilatation may actually be stored in the endothelial cells of coronary vessels and released under hypoxic conditions as part of a pathophysiological mechanism to increase coronary blood flow to prevent further damage to the heart. It can also be envisaged that interactions between release from perivascular nerves and endothelial cells could provide additional homeostatic control under normal conditions.

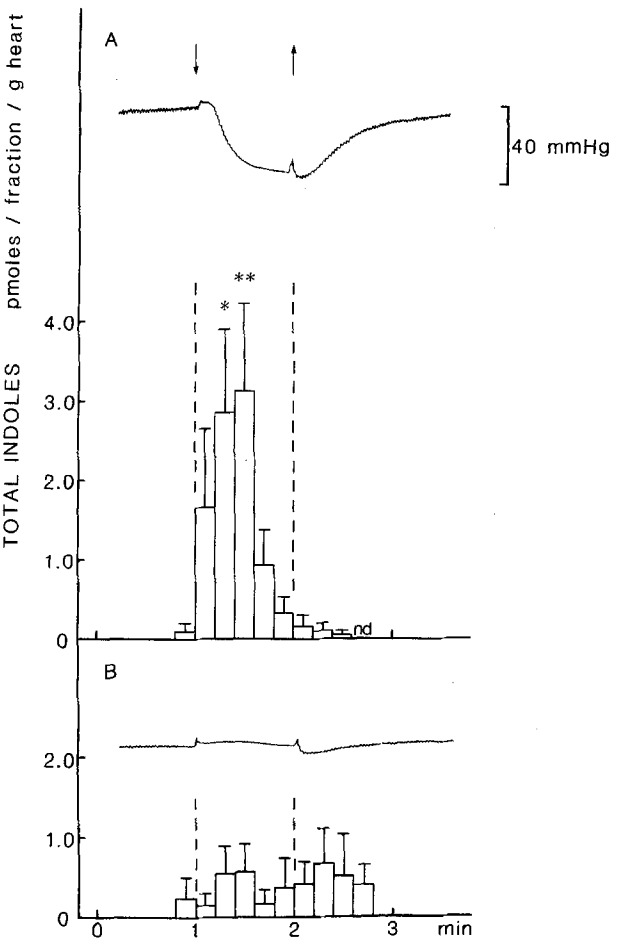


Figure 2. Release of 5-HT + 5-HIAA in rat Langendorff heart preparations and the effect of hypoxia. Arrows indicate the period when the preparations were switched to a second perfusion system. Results are given as the mean ± SEM.

*A* Hypoxia: second perfusion system contained hypoxic Krebs solution. A typical vasodilatory response of the heart to hypoxia is shown. Levels of 5-HT and 5-HIAA released reached a peak at the time of maximum vasodilatation (n = 8).

*B* Normoxic controls: second perfusion system contained normoxic Krebs solution. A typical trace shows no vasodilatory response and levels of 5-HT and 5-HIAA released were similar throughout the course of the experiment (n = 6).

Statistical comparisons were made between equivalent fractions from hypoxic hearts and normoxic controls using Student's one-tailed t-test. Levels of 5-HT and 5-HIAA were significantly higher in the perfusate from hypoxic hearts, * $p < 0.05$, ** $p < 0.025$. nd, not detectable.

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# Differential protection of radiation-induced DNA single-strand breaks and cell survival by solcoseryl[1,2]

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*Summary.* V79 Chinese hamster cells were studied in vitro for modification of cobalt-60 gamma radiation effects by solcoseryl. This treatment did not modify cell survival but did protect against DNA single-strand breaks.
*Key words.* Radiation effects in vitro; cell survival; solcoseryl; actihaemyl; DNA single-strand breaks; radiation protectors.

Solcoseryl is a deproteinized extract of calf serum which has been shown to protect mice from experimental cleft palate formation[5], to promote wound healing[6] and to modify radiation response both in the clinic[7,17] and in experimental animals[8,12,16]. The mechanism(s) of action of this agent has not been determined. Solcoseryl's ability to improve wound healing may involve stimulated formation of granulation tissue or improvement of tissue oxygenation[18,19].

The present study tested the effect of solcoseryl in vitro using V79 cells. The ability of this agent to modify cell survival after cobalt-60 gamma irradiation was studied. In addition, the effect of solcoseryl on DNA single-strand break formation after irradiation was tested using the alkaline elution technique.

*Cell preparation.* V79-B310H Chinese hamster cells were cultured at 37 °C in a monolayer on 100-mm plates in MEM-10 (Gibco) containing 10% fetal calf serum, in a water-saturated atmosphere containing 5% $CO_2$. Cells were trypsinized (0.025% trypsin in PBS), at 37 °C for 10 min. A dilution of the suspension was counted by using a Coulter counter with appropriate corrections for coincidence.

*Solcoseryl.* Solcoseryl was obtained from the manufacturer as amber liquid in sterile 2-ml ampoules. Each ml contained 40 mg of the active material. Toxicity studies indicated that the addition of up to 60 mg/plate did not modify clonogenicity of this cell line. One million cells were diluted 1:3 with solcoseryl and media, which resulted in a final concentration of 13.3 mg/ml. The cells were treated for 30 min prior to and during irradiation.

*Irradiation.* A cobalt-60 gamma irradiator (AECL, Gamma Beam 650) at a dose rate of 10 Gy/min was used for these experiments. Radiation doses included 0, 2, 4, 6, 8, 10, 12 and 14 Gy. Both treated and control cells were irradiated, at the same time, in individual 15-ml sterile centrifuge tubes in an ice bath. Immediately after irradiation the cells were diluted and plated using standard colony forming techniques.

*Alkaline elution.* The alkaline elution technique of Kohn[9] has been described in detail elsewhere[10]. Briefly, 6–8 million cells were impinged onto a 47-mm diameter (0.8 µm pore size) polycarbonate filter. Cells were washed twice and lysed with 10 ml 2 M NaCl – 0.04 M EDTA – 0.2% sarkosyl (pH 12.1). Elution was performed in the dark with 0.1 M tetrapropylammonium hydroxide and 0.02 M EDTA (pH 12.1). The flow rate was 0.04 ml/min and fractions were collected every 90 min for 15 h. The DNA in each collected fraction and that remaining on the filter was assayed using a microfluorometric technique described in detail elsewhere[11]. The method has been demonstrated to accurately represent the kinetics of DNA elution when compared to techniques using radioactive methods[10]. The designation strand-scission factor (SSF) refers to a relative value determined as a result of the comparison of associated DNA elution curves. This value is used to characterize the relative number of DNA-strand breaks present. The SSF was determined from the relationship: $SSF = |\log((fx)/(fo))|$, where fo and fx are, respectively, the proportion of DNA retained on the filter after an eluted volume of 17.5 ml for the control and corresponding treated sample.

Figure 1 shows the survival of V79 cells irradiated with or without solcoseryl. The $D_0$'s for these curves was found to be 2.4 Gy with no significant difference between treated cells and control. A correlation coefficient of 0.96 was obtained. It is apparent from these results that in vitro radiation protection under the conditions described is non-existent.

Figure 2 shows the effect of solcoseryl modification of the DNA single-strand breaks induced by gamma irradiation. Solcoseryl significantly reduced the SSF as compared to the untreated control groups for doses greater than 4 Gy.

In vitro cell survival data reported here do not correlate with improved animal survival reported after lethal irradiation[8,12]. These results suggest that the mechanism of radioprotection noted in animal lethality experiments may result from the capability of solcoseryl to modify the post-irradiation sequelae. Barth et al.[16] tested the effect of multiple injections of solcoseryl after irradiation with 6 Gy and found significant protection but less than reported by Bauer et al.[8,12]. These findings could possibly be explained by improved repair capabilities or stimulated proliferation of critical stem cells.

The ability of solcoseryl to reduce single-strand breaks in DNA after irradiation correlates well with similar results observed after treatment with a known radiation protec-