Differential Amine Depletion from Cardiac Adrenergic Nerves by Segontin

Segontin (N-(3'-phenyl-propyl-(2'))-1,1-diphenyl-propyl-(3)-amine) (Hoechst) markedly reduces the noradrenalin content of peripheral adrenergic tissues[1,2], probably by interfering with the amine uptake mechanism in the storage granules in a way similar to that of reserpine[3]. However, even large doses of Segontin s.c. do not reduce cardiac noradrenalin by more than about 60%, as measured 4 h to 7 days after the injection[1]. Using the fluorescence microscopic method of FALCK and HILLARP[4-7], the cellular localization of the residual noradrenalin was presently studied after the administration of various doses of Segontin.

Materials and methods. Albino mice of either sex, weighing about 20 g, were used. Groups of animals (6–8 in each group) were given Segontin s.c. in single doses of 10, 20, 40, 60, 80, and 100 mg/kg body weight. All animals were killed 24 h later and the hearts were taken out. The hearts from 10 untreated controls were also analyzed. The atria and the ventricles were then frozen separately to the temperature of liquid nitrogen, freeze-dried, treated in formaldehyde gas at + 80 °C for 1 h, sectioned (6 μ), and prepared for fluorescence microscopic analysis, all according to FALCK and OWMAN[8]. Under the conditions described, the primary catecholamines emit a green to sometimes yellow-green fluorescence, whose intensity, within certain limits, corresponds to the amine content[9].

In order to exclude the possibility of any fluorescence of Segontin interfering with the fluorescence of the catecholamines in the heart, 2 different model experiments were performed. A solution of Segontin or noradrenalin (Nor-Exadrin concentratum, Astra) was added to a 1% aqueous solution of human albumin (Kabi) until the final concentration of the drug was 1 mg/ml. Spots (1 μl) of the 2 final mixtures, and of albumin alone, were placed on slides, dried at room temperature and treated for 20, 40, or 60 min in formaldehyde gas at + 80 °C for fluorescence microscopic analysis[5]. In another experiment, a small amount of Segontin solution (50 mg/ml or 0.5 mg/ml) was deposited in pieces of fresh ventricular muscle tissue or cerebellar parenchyma from mice. The specimens were freeze-dried and subsequently processed for fluorescence microscopy in the same way as the above-mentioned tissue preparations.

Results. Fluorescence microscopy of the atrial and ventricular portions of the normal mouse heart revealed systems of intensely green-fluorescent adrenergic nerve terminals[10-12]. In the atria the adrenergic nerves were fairly numerous, less so in the right ventricle, and comparatively sparse in the left ventricle. The terminals were arranged both around blood vessels and in intimate contact with the cardiac muscle cells in a way suggesting innervation of both components (Figure 1a). In favorable sections, the nerves also in the heart muscles were seen to form a characteristic ground-plexus. No fluorescent perikarya were present at any site in the heart; however, large

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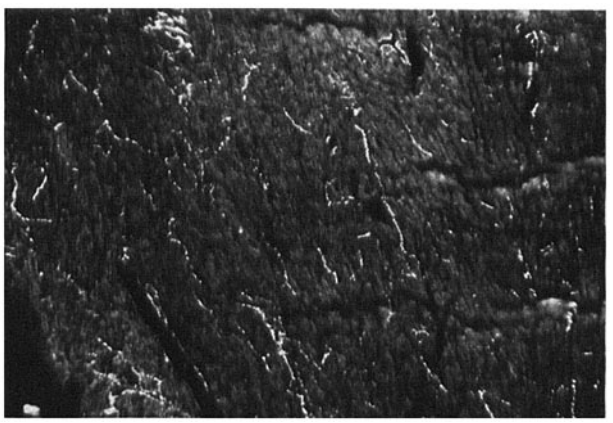
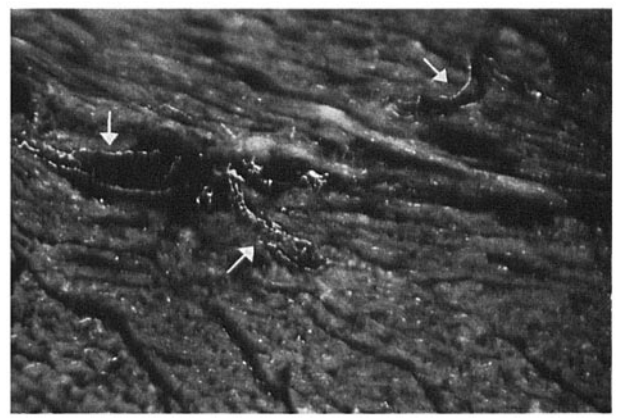


a

b



c

Fig. 1. Fluorescence photomicrographs of heart ventricles. Noradrenalin in fluorescent nerves. Small, autofluorescent pigment granules may be seen in the muscles. × 140. (a) Untreated; fluorescent nerve terminals to both vessels and muscles. (b) Segontin 20 mg/kg; same arrangement and intensity of nerves as in (a). (c) Segontin 60 mg/kg; fluorescent terminals with slightly reduced intensity only to vessels (arrows).

clusters of non-fluorescent, probably cholinergic, nerve-cell bodies were located near the pericardium of the right atrium.

Administration of 10 mg/kg or 20 mg/kg of Segontin had no overt effect on the number or fluorescence intensity of the cardiac adrenergic nerves (Figure 1b). Injection of higher doses of Segontin caused a distinct reduction in the nerve fluorescence, the reduction being equal in the atrial and ventricular portions. The findings are summarized in Figure 2. Thus, at 40 mg/kg the number of fluorescent muscular nerves was somewhat reduced, and their intensity was distinctly weaker than in the controls. However, the number of vascular nerves was unaffected, althought in some animals they fluoresced somewhat less intensely than in the untreated control animals.

A slight, but clearly visible, reduction in the number of fluorescent vascular nerves was noted at the 60 mg/kg level; their fluorescence intensity was moderate. This dose of Segontin usually abolished all the fluorescence of the adrenergic nerves to the heart muscle (Figure 1c). In only a few animals did any fluorescent muscular nerves persist; they were sparse, however, and emitted only a faint light. A further slight reduction in the number and fluorescence of the adrenergic nerves innervating the cardiac blood vessels was seen at 80 mg/kg. Essentially the same net result was obtained with 100 mg/kg of Segontin. No fluorescent nerves were demonstrable in the cardiac muscle tissue after administration of these 2 larger doses.

In neither of the model experiments did Segontin develop any fluorescence beyond the slight unspecific fluorescence of the background, while noradrenalin (in the nerves or in the albumin spot) exhibited an intense green to yellow-green light.

Discussion. The number and fluorescence intensity of the cardiac adrenergic nerves found in normal animals and after administration of varying doses of Segontin is in quite good agreement with the levels of cardiac noradrenalin measured fluorimetrically in corresponding experiments in rats[1]. In none of the model experiments did Segontin develop any fluorescence upon formaldehyde treatment. Thus, the green fluorescence in the nerves of the mouse heart in the various experiments seems to reflect the presence of a primary catecholamine[8], probably noradrenalin.

The present data suggest that the bulk, if not all, of the noradrenalin persisting in the heart after the injection of certain doses of Segontin[1,2,13] occurs in the adrenergic nerves to the blood vessels rather than to the muscle tissue of the heart. This distribution of transmitter might reflect a different sensitivity of the vascular and muscular adrenergic nerves to Segontin, or different basic properties of the nerves[14], such as a lower rate of turnover[15] in the vascular nerves as compared with the muscular adrenergic nerves of the heart. One mechanism by which Segontin can relieve angina pectoris[16] could hence be the combined effect of a preserved physiologic vasodilatation in the cardiac vascular bed in response to the release of transmitter from the vascular adrenergic nerves, and of a reduced myocardial metabolism[17] due to previous 'chemical denervation' of the heart muscle by Segontin.

Segontin is currently used in attempts to control ventricular fibrillation during induced hypothermia: Fluorescence microscopy revealed that in a hibernating animal (hedgehog) the cardiac adrenergic nerves are distributed mainly around the blood vessels, whereas few, if any, adrenergic nerves occur in relation to the muscle fibers[18]. On the basis of these and the present findings, Segontin (60 mg/kg s.c.) was injected into cats in order to obtain a similar distribution of transmitter in the cat heart as that in the hedgehog heart. Thus pretreated, the cats could be cooled to 17.8–17.2 °C, and subsequently rewarmed, without developing ventricular fibrillation[18], the major complication during induced deep hypothermia.

Conclusion. The residual noradrenalin known to persist in the heart after Segontin administration seems to be located in the vascular adrenergic nerves, whereas the muscular nerves are depleted of their transmitter. The significance of the findings is discussed[19].

Zusammenfassung. Selbst bei hohen Dosen von Segontin wird eine beachtliche Menge von Noradrenalin im Herz zurückgehalten. Fluoreszenzhistochemische Untersuchungen nach verschiedenen Dosen von Segontin machen wahrscheinlich, dass dieser Aminrest in den Gefässnerven enthalten ist, während der vollständige Schwund der Überträgersubstanz in den Muskelnerven beobachtet wird.

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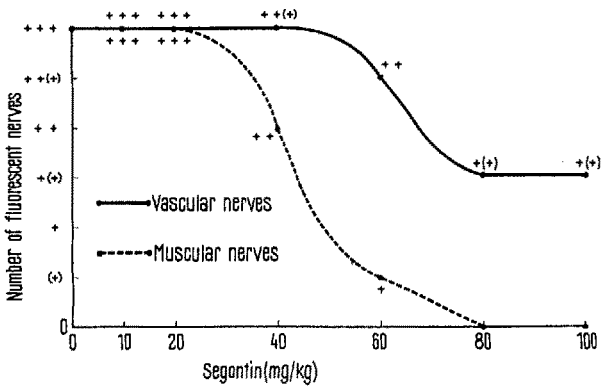

Fig. 2. Changes in number and fluorescence intensity of muscular and vascular adrenergic nerves of the mouse heart after various doses of Segontin. Number (ordinate) and fluorescence intensity (at each reading in the 2 curves) is arbitrarily expressed from 0 to +++ (maximal number or intensity). Each value is the mean of a survey of the atria and ventricles from all animals in the different groups.

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