COURSE OF WOUND HEALING IN SPONTANEOUSLY HYPERTENSIVE RATS UNDER THE INFLUENCE OF MORPHINE, SUBSTANCE P, AND ITS SP_{1-4} FRAGMENT

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Despite definite differences in the course of wound healing in hypertensive patients, this problem has not yet been studied, and as a result, a differential approach to the treatment of traumatic and other injuries in these patients is difficult. It has been shown [7, 12] that neuropeptides, including substance P (SP), are involved in the regulation of the function of the cardiovascular system and are responsible for the pathogenesis of hypertension in spontaneously hypertensive rats (SHR). These same peptides regulate repair processes in the body. Ligands of opioid receptors affect growth and development of nerve structures [6] and wound healing [1, 2] and play cytoprotective role in foci of injury and in developing tissues [4, 10]. Besides performing the function of a pain neurotransmitter, SP is also involved in various autonomic responses and it is a neurotrophic factor and an important component of tissue regeneration [8, 13]. Physiological interconnection between SP and activity of opiate receptors of cell membranes [9, 11], on the one hand, and their role in maintenance of the hemodynamics and of tissue regeneration, on the other hand, suggest that the nociceptive and antinociceptive systems are directly concerned in the maintenance of structural homeostasis, and that their humoral components may be useful for correcting the course of wound healing, in particular, in hypertensive animals.

The aim of this investigation was to study the particular features of the course of healing of skin wounds in SHR and to compare them with their normotensive control (Wistar-Kyoto rats; WKY). The second aim was to study the effect of morphine, the classical receptor antagonist of opiate receptors, and also of SP and its N-terminal fragment SP_{1-4} on the cellular basis of repair in these lines of rats.

TABLE 1. Blood Pressure (BP; M ± m, n = 10) of SHR and WKY Rats in Different Age Periods

Age, weeks	WKY		SHR	
	BP, mm Hg		BP, mm Hg	
	systolic	diastolic	systolic	diastolic
10	126±7	73±10	152±10*	96±10
20	131±6	83±4	191±11*	122±9*
30	139±8	80±5	190±15*	121±5*

Legend. *p < 0.05.

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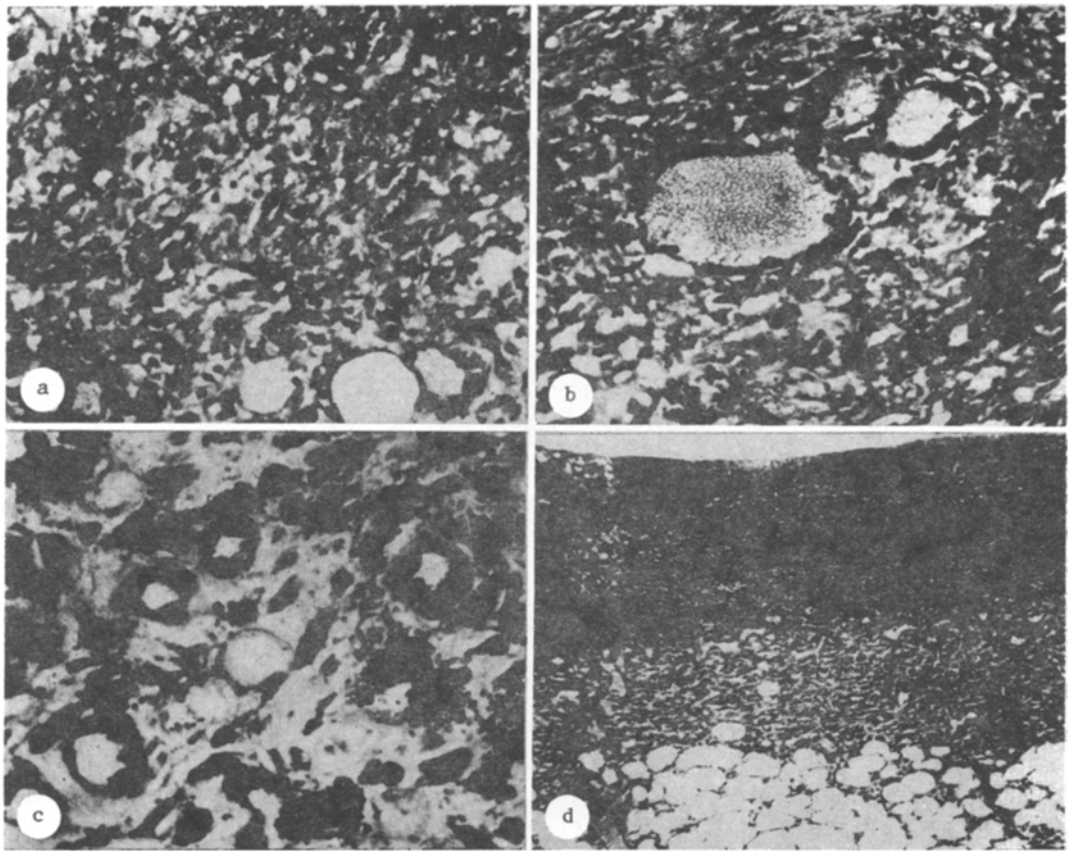

Fig. 1. Morphological picture of healing skin wounds in WKY and SHR rats. a) WKY, 3rd day: foci of GT in floor of wound; proliferation of fibroblasts and individual newly formed capillaries can be seen; weak neutrophilic infiltration; b) SHR, 3rd day: marked fibrinous exudation in tissues of wound floor; stasis and sludging of erythrocytes in lumen of venules; migration of neutrophils and monocytes through their walls; c) SHR, 3rd day: numerous mast cells, some of them degranulated; edema and cellular infiltration of tissues; d) SHR, 5th day: marked fibrinous-leukocytic layer in region of wound floor: poorly developed GT. Stained with: a, b, d) hematoxylin and eosin; c) toluidine blue. Magnification: a, b) 200; c) 400; d) 100.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male SHR and 40 WKY rats aged 10 weeks, weighing initially 250-280 g, obtained from the nursery of the Institute of Biologically Active Compounds, Academy of Sciences of the GDR. The animals were kept on a standard diet with free access to water and food and with a 12-h daylight and 12-h darkness cycle. The blood pressure (BP) was measured daily in the anesthetized rats (10 rats of each line) in the tail by means of automatic BP recording device. Under pentobarbital anesthesia (35 mg/kg intraperitoneally) a skin flap 17 mm in diameter was removed in the dorsal region of all the animals except 20 whose BP was measured. The rats of each line were divided into four groups, with 10 in each group, and for 3 days before the operation and 43 days thereafter they received the following intraperitoneal injections: group 1 – physiological saline; groups 2 and 3 – SP_{1-11} and SP_{1-4}, respectively (synthesized by Dr. Binnert and coworkers, Academy of Sciences of the GDR), in doses equimolar to the mean effective dose of Met-enkephalin [1] – 125 and 53 μg/kg body weight; group 4 received morphine hydrochloride in a dose of 2.3 mg/kg (100 times greater than the equimolar dose of Met-enkephalin). On the 3rd, 5th, and 7th days after the operation, at the same times during the morning, three rats of each group received an intraperitoneal injection of colchicine (1.5 mg/kg) to arrest mitosis in metaphase, and 4 h later, under ether anesthesia, areas of the skin wounds were excised at all depths down to the muscular fascia, and the animals were taken out of the experiment. The tissues were fixed in Carnoy's solution and transferred into 96% alcohol. Paraffin sections were stained with hematoxylin and eosin, with picrofuchsine by Van Gieson's method, by Weigert's method

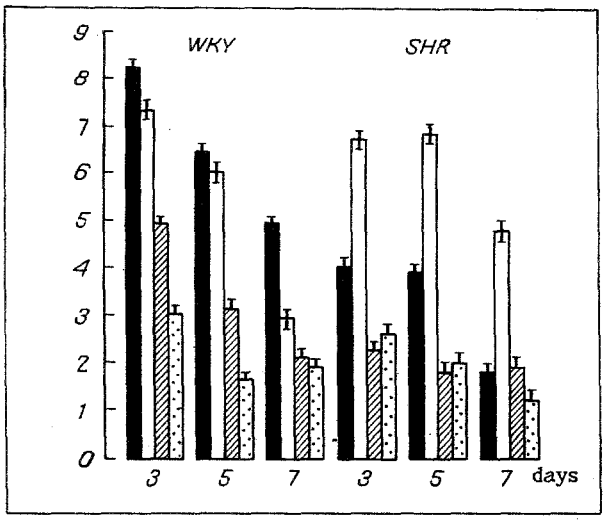

Fig. 2. Time course of mitotic activity of fibroblasts and endothelium of newly formed GT of skin wounds in WKY and SHR rats under the influence of morphine (black columns), SP_{1-11} (unshaded columns), SP_{1-4} (obliquely shaded columns), and physiological saline (dotted columns).

for elastic fibers, by Gomori's silver impregnation method, and with toluidine blue for acid glycosaminoglycans (GAG); the PAS reaction was carried out for glycoproteins, Brachet's reaction for RNA, and Feulgen's reaction for DNA. The mitotic index of fibroblasts and endothelium of the capillaries in the newly formed granulation tissue (GT) also was studied.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that by the 10th week the SHR had developed hypertension, and by the 20th week this had stabilized at a mean level of 190/120 mm Hg. Consequently, at the time of the operation on the hypertensive rats their BP was 20% higher than that of normotensive animals.

The morphological study of preparations of the skin wounds obtained by histochemical methods showed that definite differences were found between the SHR and WKY rats both in their response to wound trauma and in the action of the substances studied. On the 3rd day after the operation, GT in WKY rats was visible only in the form of discrete foci in the fatty areolar tissues, no vertical capillaries were present, there were only very few immature argyrophilic collagen fibers, and the inflammatory reaction was moderate (Fig. 1a). In the SHR, although the degree of development of GT was the same, the much more marked exudative manifestations were noteworthy: edema, fibrinous exudation, increased permeability of the walls of the capillaries and venules for leukocytes (Fig. 1b). Congestion of the vessels and aggregation of erythrocytes in their lumen were intensified, and microthrombi formed in some places. Mast cells, including degranulated forms, were more numerous (Fig. 1c). The mitotic index of the fibroblasts in the SHR was somewhat lower (Fig. 2) and mitotic figures were found less frequently in epithelial and endothelial cells.

By the 5th and 7th days, because of continued manifestations of exudation, proliferative processes in the SHR rats were less intensive than in WKY rats, their GT was less well developed, and epithelization of the defects less conspicuous (Fig. 1d).

In WKY rats, morphine, SP_{1-11}, and SP_{1-4} had a marked stimulating effect on repair processes during wound healing; the character and degree of activity of the substances, moreover, were similar. Compared with the control animals receiving physiological saline, on the 3rd day proliferative processes were intensified in GT, and the number of mitoses was increased in the fibroblasts and capillary endothelium, especially in the pericytes (Fig. 2, Fig. 3a). Fibroblasts had an increased RNA content in their cytoplasm and DNA content in their nucleus, and they contained more acid GAG and fuchsinophilic collagen fibers. The number of newly formed vessels in the maturing GT was considerably increased and vertical capillaries appeared (Fig. 3b); in the epithelium there were many cells with mitoses

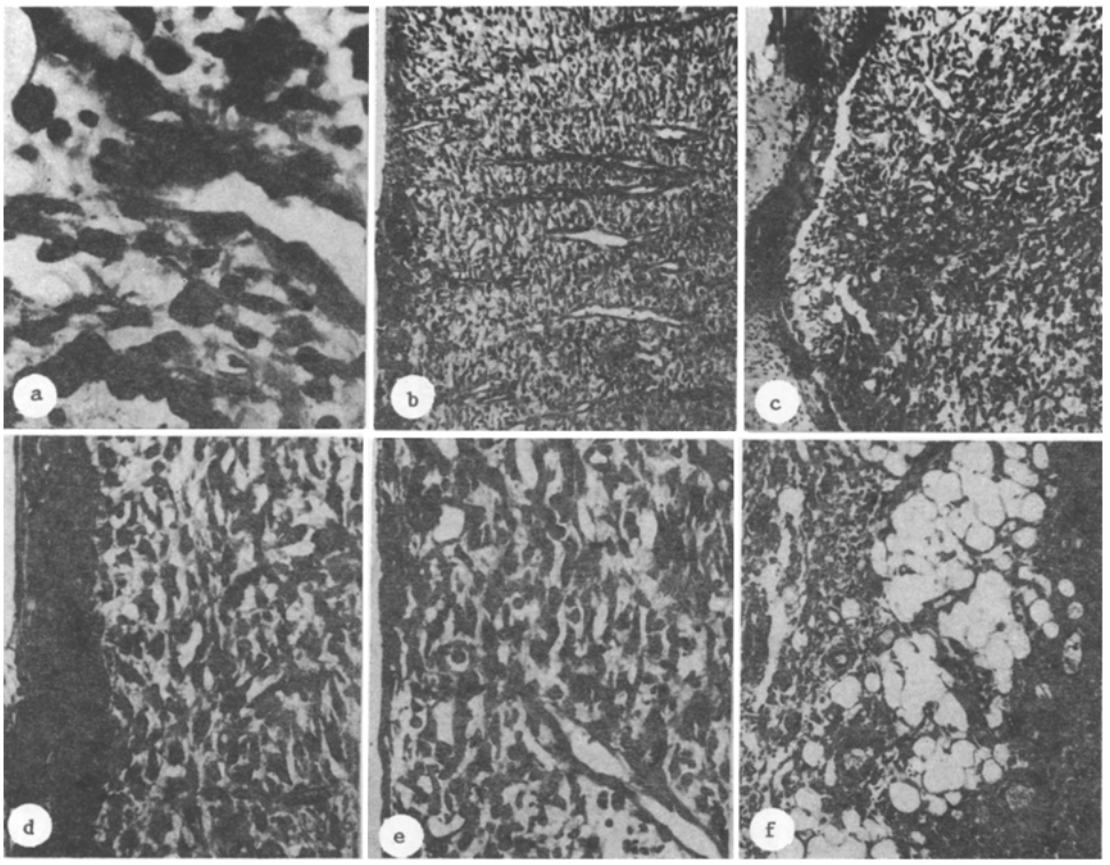

Fig. 3. Effect of substances on repair processes during healing of skin wounds in WKY and SHR rats. a) Morphine, WKY, 3rd day: proliferation of fibroblasts, new capillary formation, numerous mitoses in fibroblasts and pericytes; b) SP_{1-11}, WKY, 3rd day: relatively mature GT with vertical capillaries; c) SP_{1-4}, WKY, 3rd day: peripheral regeneration of epithelium, "creeping" of epithelial layer above well developed GT; d) morphine, WKY, 7th day: marked epithelization of wound surface, mature GT; e) morphine, SHR, 3rd day: relatively mature GT with vertical vessels; f) SP_{1-4}, SHR, 3rd day: weak development of GT in region of wound floor, marked exudative processes. Stained with hematoxylin and eosin. Magnification: a) 400; b, c, f) 100; d, e) 250.

and the content of DNA and glycogen was increased. By the 5th-7th days, more mature GT had formed in the experimental animals than in the control, and the GT contained more collagen fibers. The number of mitoses was reduced due to differentiation of the fibroblasts and cessation of active growth of the capillaries. Differentiation of the epithelium was intensified (Fig. 3d).

In SHR under the influence of morphine and SP_{1-11} marked intensification of proliferation was observed: in all the animals a continuous layer of quite mature GT with vertical capillaries and horizontal fibroblasts had formed (Fig. 3e), their content of GAG and, in particular, a fuchsinophilic collagen fibers, was high. Exudative manifestations were less marked than in the control, and peripheral epithelization was on a significantly larger scale. On the 5th and 7th days, development of GT in these groups was still at a higher level than in the control.

In SHR receiving SP_{1-4}, GT development at all times of observation was virtually identical with that in the control animals, and in some it was actually retarded. On the 3rd day after the operation GT was poorly developed (Fig. 3f), it was present only in small foci, inflammatory processes were well defined, but peripheral epithelization was slight. The mitotic index of the fibroblasts was within the control limits (Fig. 2). Later, maturation of GT was considerably retarded compared with GT in rats receiving morphine and SP_{1-11}. The absence of a stimulating effect of SP_{1-4} in SHR by contrast with WKY rats was evidently due to

a disturbance of expression of receptors for SP fragments under the conditions of genetically determined modification of metabolism of vasoactive neurotransmitters.

The main difference in the course of wound healing in SHR is thus a marked increase in capillary permeability at the sites of injury, a result either of the hypertensive state itself or of an imbalance between several neurotransmitters and hormones. Increased membrane permeability of the skin fibroblasts of SHR has been observed by other workers also [14]. The authors cited consider that disturbance of cationic transport through cell membranes may be linked with the pathogenesis of hypertension. The high level of vascular permeability, of aggregation of erythrocytes and of thrombus formation disturb the microcirculation of the tissues and their nutrition, thus prolonging the phase of inflammation and delaying the phase of regeneration. Administration of exogenous SP and morphine prevents the development of exudative phenomena in SHR and thus facilitates more rapid healing of skin wounds.

The morphological picture of the effect of morphine and SP on repair processes described above is in full agreement with the character of the effect of the opioid peptide dalargin [2], so that a common mechanism of their action can be suggested. The triggering factor of this mechanism is the nociceptive impulse, inducing release of the pain mediator SP from primary afferent neurons, and in turn, this mobilizes the system of endogenous opioids, both central and peripheral. This type of possible interaction of SP and opioids is confirmed by many facts: nociceptive stimulation leads to the accumulation of metenkephalin in the spinal cord, whereas division of afferent fibers prevents this effect [3], SP stimulates opioid peptide release in vivo and in vitro [9], opiate ligands inhibit SP release from sensory neurons [11], and enkephalins inhibit the depolarizing effect of SP in motoneurons [15]. Hence it is clear that under the influence of pain-induced stress not only is stressor analgesia formed, but also, considering the high reparative activity of enkephalins [1], the regeneration system is triggered. Evidence of the mediator role of SP in healing processes is given by data showing that exhaustion of SP from afferent neurons by capsaicin leads to an increase in wound formation arising spontaneously and induced by injection of HCl, and to delay of healing [8]. The SP concentration in the skin falls considerably during regeneration [13]. The effect of SP may be due, at least partially, to its ability to mobilize histamine and serotonin – important regulators of the local hemodynamics, and also to exert an immunomodulating effect [12].

Synergism in the character of the effect of the functional antagonists, opiates and SP, on wound healing confirms the suggestion put forward previously that pain plays an important role as inducer of the combination of mechanisms that lie at the basis of regeneration of injured tissues.

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