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A new approach to analysis of human sweating

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Abstract. In human skin transplanted to the back of 3 strains of immuno-deficient mice the function of the eccrine sweat glands of the human transplant was tested by topical intradermal application of pilocarpine, adrenaline and atropine + pilocarpine. Sweat responses were observed in pre-selected fields of observation by means of video macroscope. The iodine starch reaction served as an indicator for the appearance of sweat sport and permitted the evaluation of areas wetted by sweat in the field of observation. Among 9 animals tested, the hybrids between the CB-17-*scid* mouse and the BALB/cA-*nu* mouse (BALB/cA-*nu,scid*) seemed to exhibit the most consistent sweating response to local pharmacological stimulation. According to histological examination, eccrine sweat glands were preserved in human skin transplanted into the back skin of the BALB/cA-*nu,scid* mouse strain. The heterologous, human skin graft provides a novel model permitting, independent of the normal sweat gland innervation, the analysis of molecular receptors of sweat gland cells by which the actions of natural transmitters and pharmacological agents are transduced.
Key words. Human skin; heterologous transplant; immuno-deficient mouse; eccrine sweating; pilocarpine; adrenaline; atropine.

Human eccrine sweat glands are innervated by postganglionic sympathetic nerve fibers with acetylcholine as the predominant neuro-effector transmitter. Accordingly, sweating can be induced by topical application of acetylcholine and pilocarpine. Sweat glands also receive some input from noradrenergic fibers and, hence, adrenaline is locally effective in stimulating sweat secretion[1–3]. Vasoactive intestinal polypeptide (VIP) has been identified as a transmitter of sudomotor nerve endings[4]. As a means of determining the presence of corresponding receptors in sweat gland cells, independent of their innervation, heterologous transplants of human skin may be a useful tool. The CB-17-*scid* mouse (severe combined immuno-deficiency due to T-cell and B-cell defects) and the nude mouse (lacking T-cells) are suitable recipients of transplants. The BALB/cA-*nu*, mouse (*nu* = nude) and the BALB/cA-*nu,scid* mouse (hybrid of CB-17-*scid* mouse and BALB/cA-*nu*, mouse) were developed as recipients for human skin grafts at the Central Institute for Experimental Animals in cooperation with the Kanagawa Academy of Science and Technology (KAST) and the School of Medicine, Tokai University[5], and also appeared suitable for the analysis of sweating[6]. The present study exploits the successful transplantation of human skin onto the back skin of individuals of three of the above immuno-deficient mouse strains: CB-17-*scid*, BALB/cA-*nu*, and BALB/cA-*nu,scid*. No reports seem to exist about sweat gland function in heterologous human skin grafts. Therefore, sweat gland function was investigated to get some basic information from this model about secretory capability and neurochemical control maintained by human sweat glands.

Figure 1. Appearance of a BALB/cA-*nu,scid* mouse with a heterologous skin transplant (human skin).

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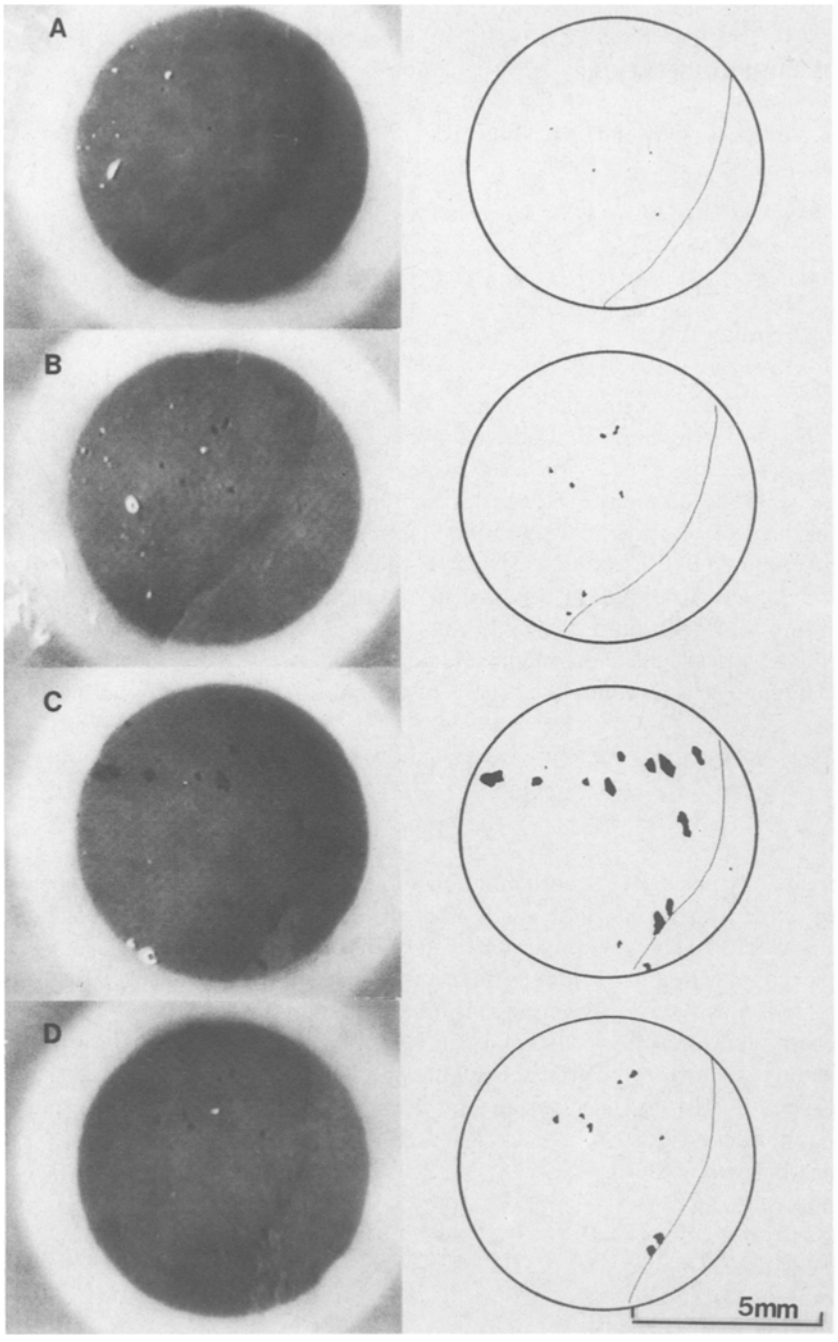

Figure 2. Fields of observation of transplanted skin viewed by the video macroscope and printed out by a video printer (Panasonic, VW-VAS 100).
Left: Aspects of the skin surface with differently developed sweat spots after injection of *A*) isotonic saline as control, *B*) adrenaline, *C*) pilocarpine, *D*) atropine + pilocarpine.
Right: Schematic evaluation of sweat spots and sizes of wetted areas in the corresponding fields of observation.

Methods

Animals. Altogether nine mice from the characterized strains were used:
a) Four CB-17-*scid* mice aged 24–29 weeks, weighing 15–27 g,
b) Four BALB/cA-*nu,scid* mice 29 weeks, weighing 20–23 g, and
c) One BALB/cA-*nu* mouse aged 29 weeks and weighing 18 g. To all animals human skin had been transplanted into the dorsal integument, as illustrated by figure 1, in collaboration with KAST and the School of Medicine, Tokai Univeristy. Experiments were carried out 17–20 weeks after transplantation. Upon arrival in the Institute of Tropical Medicine, the mice were kept in single cages in a conventional animal room at 26 °C ambient temperature. Experiments were done within a week. The animals were maintained for a further month and remained in good condition.

Experimental procedure. The experiments were carried out in an enivironmental chamber at 26 °C ambient temperature and 60% relative humidity. The animal was gently held on a board by fixing the extremities with soft adhesive tape. This procedure and the subsequent

studies were approved by the Animal Care and Use Committee of the Central Institute for Experimental Animals. Sweating was monitored by using the iodine-starch reaction (Minor's method): application of iodine dissolved in absolute alcohol to the skin was followed by starch powder suspended in castor oil. A skin area of 0.5 cm^2 was observed with a video macroscope (Nihon Kohden, VMS-1300) showing the magnified skin area (see fig. 2) on a monitor. For quantitative evaluation the number of sweat spots emerging from the iodine-starch reaction in a given observation field were counted. Further, the video image was copied (Panasonic, VW-VAS100), magnified 1.8-fold, and the wetted areas displaying the iodine-starch reaction excised from the copy, weighed and their surface calculated in mm^2 from the known paper weight per unit area. As stimulants for the sweat glands the following agents were dissolved in isotonic saline: adrenaline (1.0 mg/ml), pilocarpine (10 mg/ml), atropine (0.1 mg/ml), and VIP (0.33 mg/ml). Quantities of 0.01 ml of the dissolved agents were injected intracutaneously with a 26 G needle inserted at an angle of 15° to a depth of 2 mm and at a distance of 5–8 mm from the observation area.

Table 1. Comparison of number of sweat spots and size of wetted area induced by different pharmacological stimulants in human skin graft transplanted into the back skin of a BALB/cA-*nu,scid* mouse.

	Chemical stimulant	Number of sweat spots	Area wetted by sweat (mm^2)	Ratio
A	Control	4	0.107	1.00
B	Adrenaline	8	0.284	2.64
C	Pilocarpine	14	1.83	17.1
D	Atropine + pilocarpine	8	0.314	2.93

Each chemical stimulant was administered intracutaneously in volumes of 0.01 ml (for doses see text). *D*: pilocarpine was administered 1.5 min after atropine injection.

Control injections were isotonic saline of the same quantities. The combined effect of atropine and pilocarpine was tested by injecting the blocker 1.5 min prior to the agonist. Only one agent was tested in an animal on a single day. After the end of the experiments, the animals received an overdose of an anesthetic (pentobarbital sodium). Specimens of the skin graft were embedded in paraffin following formaline fixation. Sections perpendicular to the skin surface were cut on a microtome and stained with hematoxylin-eosin. Immuno-histochemical staining, S-100 (DAKO) was also used for detection of nerve strands of human origin.

Results

With pilocarpine as the cholinergic agonist, sweat spots started to appear within about 1 min and the wetted area subsequently increased to reach a maximum. The response to pilocarpine applied after atropine was distinctly reduced. Adrenaline caused the appearance of sweat spots after 1.5 min. With isotonic saline as the control solution sweat spots appeated no earlier than 7 min after the injection. Table 1 shows the evaluation of spot numbers and wetted areas in single experiments on a BALB/cA-*nu,scid* mouse. Number of sweat spots and wetted area appear to be positively correlated, but the limited number of data pairs does not allow correlation analysis. Qualitatively, the following rank order of efficiency was found, as illustrated by figure 2: pilocarpine > atropine + pilocarpine > adrenaline > control solution.

Table 2 lists the observations made in the nine investigated animals. While the BALB/cA-*nu* mouse and three out of the four CB-17-*scid* mice did not respond, one or the other agent was effective in one CB-17-*scid* mouse in all four tested BALB/cA-*nu,scid* mice. Taking pilocarpine as the most efficient agent, four positive and five negative trials were obtained in nine experiments.

Table 2. Summary of observations obtained by injecting different pharmacological agents into human skin transplanted into the back skin of nine individuals from three different strains of immuno-deficient mice.

Chemical stimulant / Animal	Adrenaline			Pilocarpine			Atropine + Pilocarpine			VIP
	(re)	(spots)	(area)	(re)	(spots)	(area)	(re)	(spots)	(area)	
CB-17-*scid*	−							***		***
CB-17-*scid*	+	4	0.109	+	5	0.103	−			***
BALB/cA-*nu,scid*	+	8	0.284	+	14	1.83	+	8	0.314	***
BALB/cA-*nu,scid*	+	3	0.038	−			−			−
CB-17-*scid*	−			−			−			−
BALB/cA-*nu,scid*	−			+	4	0.077	−			−
BALB/cA-*nu*	−			−			−			***
BALB/cA-*nu,scid*	−			+	13	0.098	−			−
CB-17-*scid*	−			−			−			***

(re) = response (appearance of sweat points);
(+) positive, (−) negative;
(spots): number of sweat spots;
(area): wetted area [in mm^2];
*** not investigated.

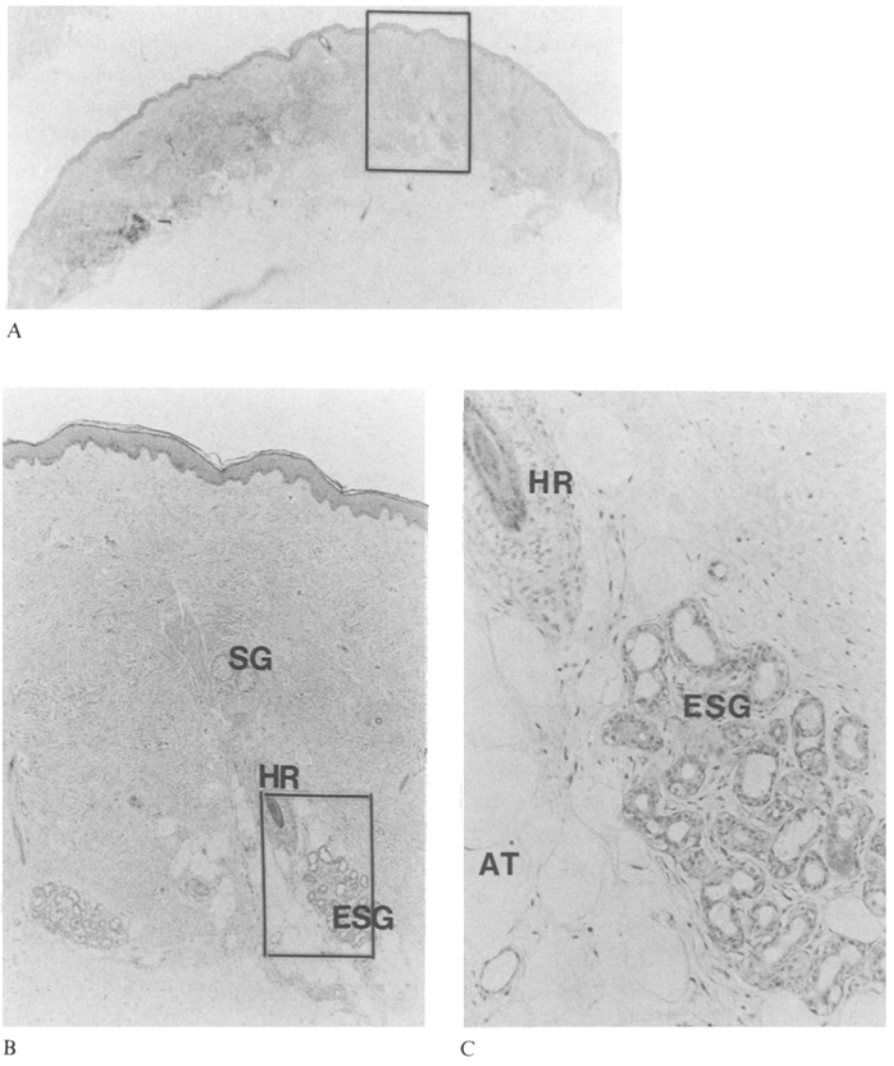

Figure 3. Histological sections (hematoxylin-eosin stain) from human skin transplanted into the dorsal skin of a BALB/cA-*nu,scid* mouse. The sections are taken from a skin area where the appearance of sweat spots were observed. HR: hair root; SG: sebaceous gland; AT: adipose tissue; ESG: eccrine sweat gland showing no signs of degeneration. Magnification: *A*) ×2.5; *B*) ×10; *C*) ×25.

Adrenaline was effective in three out of nine trials. With pilocarpine in combination with atropine only one out of eighth stimulations was effective. No positive response was observed with VIP.

The result of the histological examination is shown in the example of figure 3, taken from a BALB/cA-*nu,scid* mouse. The human skin grafts were well attached to the recipient's skin with no signs of inflammation, although with some degree of fibrosis in the transplanted dermis. Eccrine sweat glands shown in the sections of figure 3 appear well preserved. The average distance between glands corresponded to that of sweat points on the surface of the dermis. Not indicated in the figure are occasional traits of degenerated skin vessels and nerve strands of human origin. Compared with the results of figure 3, sweat glands were similarly well preserved in the CB-17-*scid* mouse but were degenerated in the BALB/cA-*nu* mouse.

Discussion

The functional studies on sweat gland stimulation of human skin grafts transplanted into immuno-deficient mice suggest that sweat gland function was preserved best in the animals with the most severe combined immuno-deficiencies, i.e. the BALB/cA-*nu,scid* mice. While the limited number of experiments does not permit the statistical confirmation of this impression, it is supported by the results of the histological examination according to which the morphology of human skin and eccrine glands seemed to be preserved best in the same mouse strain. Since the recipient animals do not possess eccrine sweat glands, the histologically demonstrated sweat glands are most likely of human origin, although an unequivocal identification may require the application of histocompatibility antigen in future studies.

To our knowledge, the pesent study is the first attempt to analyze the agents involved in functional stimulation of human sweat glands in a heterologous transplantation model. With the exception of VIP, each of the applied sympathetic chemical transmitters, i.e. adrenergic and cholinergic agonists, seemed to be capable of stimulating the sweat glands. Although it appears that after transplantation the muscarinic control mechanism remained dominant, the final decision must be left to dose-response studies. A closer analysis should also include VIP[4], despite the negative results of the present pilot study, and perhaps also neuropeptide Y as a transmitter which may be co-localized with noradrenalin[7].

Human sweat glands in vivo may be indirectly affected by sympathetic activities serving primarily vasomotor functions, such as vasoconstriction in the cold, due to α-adrenergic action, and vasodilatation in the heat as a result of the decrease of vasoconstrictor tone[8]. It has further been suggested that sympathetic vasodilatator innervation including the forearm may contribute to increases in skin blood flow by the activation of peptidergic component[4]. The novel approach to the investigation of human sweat glands which is provided by the model in the present study permits the simultaneous application of pharmacological and histochemical methods to study the receptor mediating secretory responses, as well as the time course of receptor dynamics after depriving the glands of their natural innervation.

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