Relationships between neural crest cells and catecholamine in suckling mice

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Summary. In suckling mice injected i.p. with a 27 mg/kg dose of L-hydrochloric acid isoproterenol, multiple neural crest tumors developed and cell death of neural crest cells occurred. It is speculated that neural crest derivatives may be β-receptor cells and contain regulatory units of neurotransmission mediated through cyclic AMP.

Neural crest cells are believed to be proprioceptive cells. It was found that neural crest cells in in suckling mice take up L-dopa, an intermediate product in the synthesis of epinephrine and melanin[1]. According to Pearse's hypothesis[2,3], they will produce dopamine as a prelude to norepinephrine production. In suckling mice injected with the sympathomimetic drug isoproterenol, which belongs to the catecholamine group of compounds, multiple neural crest tumors developed as a consequence.

Materials and methods. L-hydrochloric acid isoproterenol was used as the experimental catecholamine. A total of 50 mice of the ICR-JCL strain were injected i.p. with isoproterenol. 20 of these mice were given a dose of 27 mg/kg within 24 h after birth, and sacrificed in the course of 48 h after injection. 10 mice untreated with isoproterenol were used as controls. The control and experimental mice were prepared for histological examination. The whole bodies were fixed with 10% neutral formalin in 30 cases or with absolute alcohol in 10 cases or Helly's fixative fluid in 10 cases. The fixed bodies of the mice were cut serially into sagittal, horizontal and coronal sections at a thickness of 7 µm. The sections fixed with 10% neutral formalin were stained with hematoxylin and eosin and ferric chloride stain for melanin. Some specimens fixed with absolute alcohol were stained with Unna's polychrome methylene blue stain. Specimens fixed with Helly's fixative fluid were stained with panoptic combination methods of Pappenheim's and Giemsa's stains.

Results and discussion. In the suckling mice injected with a dose of 27 mg/kg of isoproterenol, multiple excessive cell proliferation or tumors and heterotopic melanin pigmentation were seen at the sites where neural crest cells may be present[4,5]. At these sites, a few neural crest cells which had lost their staining capacity, leading to cell death, were found scattered. The histological features of tumors or excessive cell proliferation were represented as follows: 1. An interlacing network of wavy, flowing streams of spindle-shaped cells in which the nuclei palisade. This characteristic tumor was found in the submucosa and muscularis mucosae and serosa of the stomach and the small and large intestines, in the muscularis mucosae and submucous layer and tunica adventitia of the esophagus, and in the walls of blood vessels. 2. A round-cell type, with large pale-staining nuclei forming a rosette found in the retina. 3. Cell masses consisting of intertwining bundles of spindle-shaped cells found along the aorta and pulmonary trunk and in the interatrial septum of the heart and in the pulp of the tooth. 4. A compact intertwining proliferation of uniform spindle-shaped cells, found in the peritoneum, in the periosteum, in the periodontal ligament of the tooth and in the pericardium. 5. Intertwining bundles of spindle-shaped cells with abundant fibrous tissue, found in the cranio-spinal nerves. 6. Cell groups or cell masses composed of basophil small round cells in various sizes were present. According to the tissues they showed often certain cell-types, and also the blood cells such as the basophils, small lymphocytes, macrophages, mast cells, lymphocyte or lymphocytoid plasma cells, and transitional cells were present. They were found in the parotid or submandibular glands and ducts, in the thyroid glands, in the thymus, in the medulla of adrenal glands, in the Langerhans and ducts of the pancreas, in the sinusoids of the liver, in the glomerulus of the kidney, in the alveolar ducts and branchioles of the lung, in the hypophysis, in the pineal body, in the nucleus caudatus or stratium and along the small blood vessels in the cerebrum, in the external granular layer of cerebellum, in the bone marrows and in the cranio-spinal ganglia. 7. Mastocytosis found in the oral mucosa and the skin and in certain bone marrow. 8. Melanoma found in the posterior segment of the choroid, in the skin, in the tongue, and in the oropharynx. Further, heterotopic melanin pigmentation in mucous membranes in the gastric mucosa and in the periosteum of vertebrae. The above findings resembled those found in suckling mice injected with mitomycin C[6,7], endotoxin[8] and steroid hormones[9]. In the suckling mice injected with a lower dose of L-hydrochloric acid isoproterenol 27 mg/kg, histological anomalies were rarely found. Isoproterenol is an inducer of cyclic AMP in certain cells

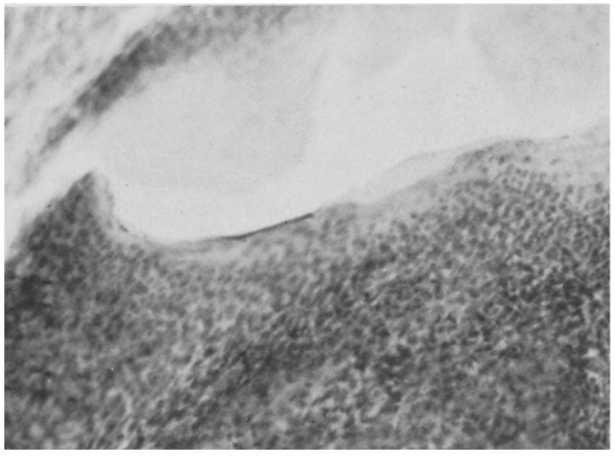

Fig. 1. The oropharynx in suckling mice injected with isoproterenol. H-E stain. × 800.

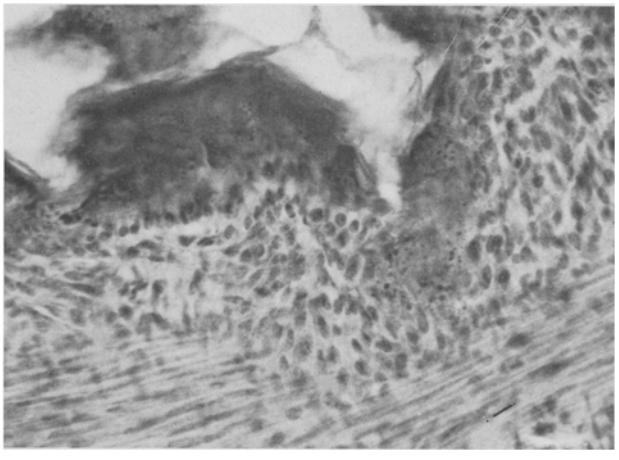

Fig. 2. The stomach in suckling mice injected with isoproterenol. H-E stain. × 800.

and it causes excessive cell proliferation in salivary glands[10], also in the liver[11] and kidney[12]. By contrast, certain cells in tissue or cell cultures are killed by isoproterenol[13]. It is thought that cell death by cytotoxic action is mediated by cyclic AMP[14], and may be related to mutation. It has become a question whether so-called DNA repair processes restore the DNA template sufficiently for DNA replication to occur and for the cell to survive, but with its DNA modified so as to lead to subsequent base sequence errors during DNA transcription and hence to mutation[15]. Cyclic AMP may have a direct effect upon the transcription but not upon the replication of DNA[16], and it can induce the undifferentiated cells to differentiate into the derivatives of the neural crest[17], possibly acting in the G phase of the cell cycle[18]. It is thought that the cells in the G phase of the cell cycle may be in the undifferentiated state[19]. It was previously reported that neural crest cells may have something to do with cyclic AMP[20,21] and they may be specific in DNA nucleotide sequences[21]. The neural crest derivatives which we referred to as 'neural crest cells' may exist in the G phase of the cell cycle[22]. The mutation or cell death of neural crest cells caused by the increased intracellular cyclic AMP resulting from stimuli such as mitomycin C[6,7,23], endotoxin[8] or steroid hormones[9,20] in suckling mice, may be related to the previous observations. On the other hand, it was observed that the satellite cells which surrounded the postganglionic neurons of bovine superior cervical ganglia demonstrated the cytochemical localization of cyclic AMP by the method of Wedner et al[24]. Intense staining could be demonstrated in small cells which did not appear to be postganglionic neurons which may be fibroblasts[25], and that mast cells in the ganglia may represent the part of another regulatory unit which modulates neurotransmission through cyclic AMP[26]. Isoproterenol is a β adrenergic agent and activation of β receptors results in an increase in cyclic AMP level[27] and β adrenergic receptors may be some nonneural elements, possibly the glia or fibroblasts[28]. Mast cells[7], chromaffin cells[4], some blood cells[22], fibroblasts[2] and the satellite cells or small cells in the ganglia may be of neural crest origin. Interestingly, catecholamine-containing cells are present in the heart[29,30], and Jacobowitz argues that they should be designated as chromaffin cells[29]. The sites of chromaffin cells in the heart correspond with the sites where the neural crest cells may be present[1] and where neural crest tumors developed in the heart[6-9]. It is thought that in suckling mice injected with isoproterenol, multiple tumors or excessive cell proliferation may be mediated by the increased intracellular cyclic AMP in the G_1 phase of the cell cycle of neural crest derivatives. Further, we wish to report here that the neural crest derivatives in the G_1 phase of the cell cycle may have a receptor and a regulatory unit of neurotransmission through cyclic AMP.

1 T. Nozue and T. Kayano, Acta anat. *97*, 114 (1977).
2 A.G.E. Pearse, J. Histochem. Cytochem. *17*, 303 (1969).
3 A.G.E. Pearse and J.M. Polak, Gut *12*, 783 (1971).
4 T. Nozue and T. Kirino, Okajimas Folia anat. jap. *50*, 183 (1973).

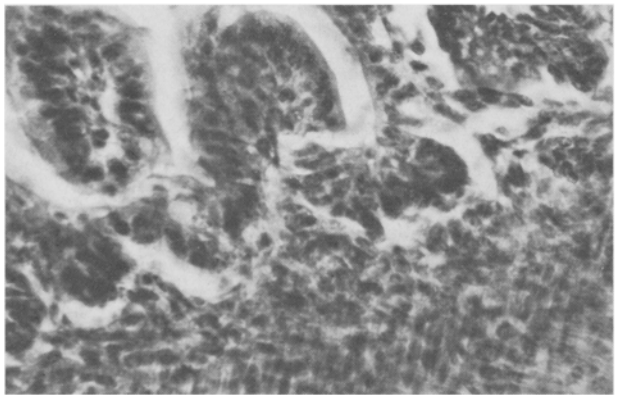

Fig. 3. The small intestine in suckling mice injected with isoproterenol. H-E stain. × 800.

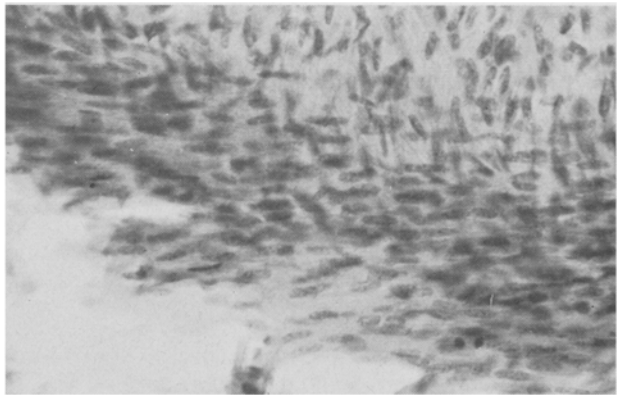

Fig. 5. The peritoneum in suckling mice injected with isoproterenol. H-E stain. × 800.

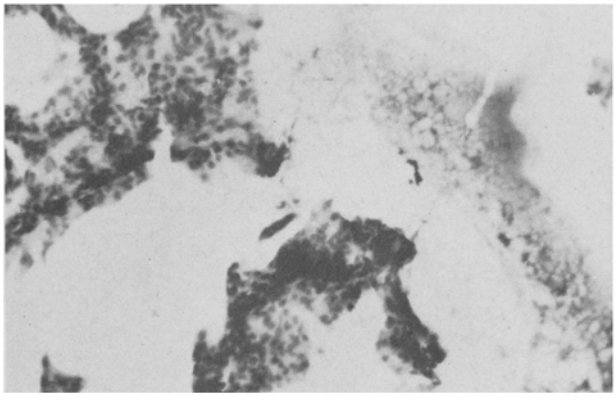

Fig. 4. The lung in suckling mice injected with isoproterenol. H-E stain. × 800.

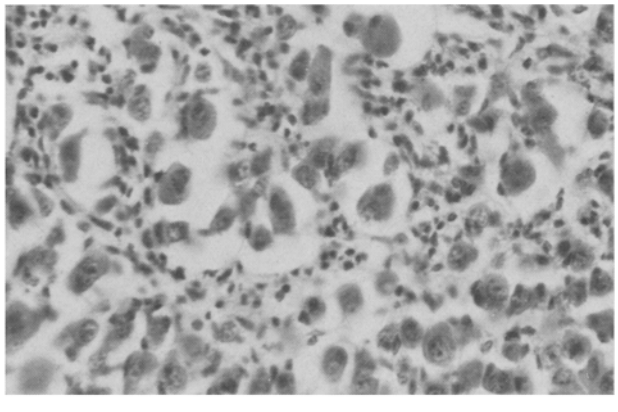

Fig. 6. A spinal ganglion in suckling mice injected with isoproterenol. H-E stain. × 800.

5 T. Nozue and M. Tsuzaki, Okajimas Folia anat. jap. *51,* 131 (1974).
6 T. Nozue, M. Tsuzaki and M. Shikimori, Experientia *30,* 1330 (1974).
7 T. Nozue, M. Shikimori and T. Kayano, Experientia *31,* 1209 (1975).
8 T. Nozue and T. Kayano, Experientia *33,* 516 (1977).
9 T. Nozue and T. Kayano, Experientia *33,* 1640 (1977).
10 H. Selye, R. Veilleux and M. Cantin, Science *133,* 44 (1961).
11 T. Barka, Exp. Cell Res. *39,* 355 (1965).
12 T. Sasaki, Igaku-no-aumi. *80,* 285 (1971). (in Japanese).
13 P. Coffino, H.R. Bourne and G.M. Tomkins, Am. J. Path. *81,* 199 (1975).
14 K.N. Prasad and J.R. Sheppard, Exp. Cell Res. *73,* 436 (1972).
15 J.J. Roberts, J.E. Sturrock and K.N. Ward, in: Chemical carcinogenesis, part A, p. 401. Ed. O.P.T. Paul and A.D. Joseph. Marcel Dekker Inc, New York 1974.
16 C.W. Abell and T.M. Monahan, J. Cell Biol. *59,* 549 (1973).
17 H.L. Wahn, L.E. Lightbody, T.T. Tchen and J.D. Taylor, Science *188,* 366 (1975).
18 J.W. Smith, A.L. Steiner, W.M. Newberry and C.W. Parker, J. clin. Invest. *50,* 432 (1971).
19 G.M. Tomkins, in: The Cell Surface: Immunological and Chemical Approaches, vol. 51, p. 138. Ed. D.K. Barry and A.R. Ralph. Plenum Press, New York 1974.
20 T. Nozue, Okajimas Folia anat. jap. *51,* 1 (1974).
21 T. Nozue and M. Tsuzaki, Okajimas Folia anat. jap. *51,* 103 (1974).
22 T. Nozue and T. Kayano, Experientia, in press, (1978).
23 T. Nozue and T. Kayano, Acta anat. *100,* 85 (1978).
24 H.J. Wedner, B.J. Hoffer, E. Battenberg, A.L. Steiner, C.W. Parker and F.E. Bloom, J. Histochem. Cytochem. *20,* 293 (1972).
25 J.W. Kebabian, F.E. Bloom, A.L. Steiner and P. Greengard, Science *190,* 157 (1975).
26 P. Kalix, D.A. McAfee, M. Schordert and P. Greengard. J. Pharmac. exp. Ther. *188,* 676 (1974).
27 H. Dramer, D.G. Johnson, I. Hanbauer, S.D. Silberstein and J.J. Kopin, Brain Res. *53,* 97 (1973).
28 T. Lindle, H. Behrendt, M.C.B. Heinl-Sawaja, E. Teufel and H. Cramer, Naunyn-Schmiedebergs Arch. Pharmac. *286,* 283 (1974).
29 D. Jacobowitz, J. Pharmac. exp. Ther. *158,* 227 (1967).
30 G.D.JR. William and C.P. Gene, Anat Rec. *177,* 265 (1974).

Binding of fluoresceinated lectins to normal and dinitrofluorobenzene treated human leucocytes[1]

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Summary. Using fluoresceinated lectins we have shown the receptor distribution on normal human granulocytes and lymphocytes following tagging with 1-fluoro- 2, 4-dinitrobenzene (DNFB). DNP-tagged cells exhibited strong, smooth membrane staining and produced smaller patches dispersed uniformly over the entire cell surface.

We previously have reported that the agglutination of dinitrophenylated normal human peripheral blood granulocytes by concanavalin A (Con A)[4] and lymphocytes by wheat germ agglutinin (WGA)[5] was qualitatively and quantitatively similar to the reactivity of human leukemic cells with these lectins[6-8]. This observation, together with our previous finding that DNP-tagged cells were capable of evoking the production of antibodies directed against human leukemia associated antigens[9-11], suggested that dinitrophenylation may have induced surface membrane changes which normally are associated with malignancy. In a recent study[12] we have quantified the binding of Con A to DNP-tagged and untagged cells and have observed that both bound equivalent amounts of the lectin. Based on these findings we concluded that the agglutination of dinitrophenylated cells by Con A was due to a rearrangement of lectin receptors similar to that which has been reported for malignant cells[13]. In the present work, using fluoresceinated lectins, we have attempted to study the receptor redistribution on DNP-tagged granulocytes and lymphocytes following binding of Con A and WGA.

Materials and methods. Granulocytes and lymphocytes were separated from blood obtained from healthy donors by means of Ficoll-Isopaque density gradient sedimentation according to the method of Boyum[14]. Cells were tagged with 1-fluoro-2, 4 dinitrobenzene (DNFB, Sigma Chemical Co., St. Louis, Missouri) at a ratio of 10^{11} molecules of DNFB per cell as described previously[4,5]. Con A (Nutritional Biochemical Co., Cleveland, Ohio) at a concentration of 100 mg/ml and a 1% stock solution of WGA prepared[15] from wheat germ lipase, type I (Sigma Chemical Co., St. Louis, Missouri) were conjugated to fluorescein isothiocyanate (FITC) by a standard method[16]. The fluoresceinated Con A (FITC-Con A) that was employed in the present study had a final molar fluorescein to protein (F/P) ratio of 1.5 and a protein concentration of 5.5 mg/ml. The fluoresceinated WGA (FITC-WGA) had a F/P ratio of 4.5 and a protein concentration of 6.0 mg/ml. These conjugat-

Binding of FITC-Con A and FITC-WGA to untagged and DNP-tagged human granulocytes and lymphocytes[c]

FITC[a]-labeled lectin	Concentration µg/ml	Intensity of membrane fluorescence[b] Untagged granulocytes	DNP-tagged granulocytes	Untagged lymphocytes	DNP-tagged lymphocytes
Con A	128	3+	2+	3+	2+
	32	1+	1+	2+	1+
	8	1+	1+	1+	1+
	2	0	0	0	0
WGA	128	2+	2+	2+	2+
	64	1+	1+	1+	1+
	32	0	0	1+	1+
	16	0	0	0	0

[a]FITC - Fluorescein isothiocyanate; [b]The intensity of membrane staining was scored on a scale of 0-4+; [c]The determinations were done by 2 independent investigators and the findings were in good agreement.