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LOCALIZATION OF PAN-CADHERIN IMMUNOREACTIVITY IN ADULT RAT TISSUES

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Cadherins, being responsible for selective cell recognition and normal tissue integrity in adults, regulate morphogenesis in a variety of organs during development. In this study, anti-rat pan-cadherin antibody, specific to all subgroups of the cadherin family, was used to map the distribution of the pan-cadherin immunoreactivity in adult rat organs. Pan-cadherin immunoreactivity positive tissues were: secretory cells of the adenohypophysis, autonomic nerve, corneal epithelium, oesophageal nerve plexus, stomach and pyloric glandular cells, epithelium of the ileum and its nerve plexus, alveolar cells of the lung, proximal convoluted tubules of the kidney, islet cells of Langerhans, and the acinar cells of the exocrine pancreas. For the first time, positive pan-cadherin immunoreactivity was demonstrated in the epithelial cells of the corpus ciliaris and in the nerve plexus of corpus cavernosum of the penis. In conclusion, our results suggest that cells in many tissues and organs of the adult rat synthesize cadherins.

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KEYWORDS: cadherin; cell adhesion molecules; rat; immunocytochemistry.
ABBREVIATIONS: IR, immunoreactivity.

INTRODUCTION

Cadherins are transmembrane proteins with 120–140 kD molecular weights, which are dependent on the presence of Ca^{2+} in structure and function. They bind cells to each other by homophilic interactions. In these interactions, they selectively bind to identical cadherin types (Behrens, 1994). Antibodies to cadherins were shown to spoil the morphogenesis of some embryonic organs *in vitro*. These observations suggest that cadherins play a crucial role in construction of tissues (Takeichi, 1988). Through their homophilic binding, cadherins bring the opposing membranes of neighbouring cells in close proximity. They are able to control the polarization of cells and may behave as ligand or receptor themselves and hence exert direct signalling activity (Vleminckx and Kemler, 1999). Thus, interaction of two cadherins on neighbouring cells could intracellularly trigger a cascade of biochemical events, which ultimately leads to the correct positioning, recognition, and communication of cells (Behrens, 1994). The suppression of cadherin activity may trigger the release of tumour cells (Takeichi, 1991). Nomenclature is according to their tissue expression.

The aim of the present study was to elucidate possible sites of cadherin formation, therefore, map the distribution of cadherin immunoreactivity in various types of cells in normal adult rat tissues.

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MATERIALS AND METHODS

Animals

Ten Wistar albino rats were used, which were 60–80 days old and 250–280 g body weight kept in a 12:12 h dark/light cycle at 22–25°C. All animals were fed *ad libitum* and had free access to water. Tissues were removed from rats after perfused under ether anaesthesia through the left cardiac ventricle with 0.9% NaCl followed by 4% parafor-

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maldehyde in 0.1 M phosphate buffer (pH 7.5) for 20 min. Perfused tissues were fixed at room temperature in the same fixative for 24 h, washed in water, dehydrated and embedded in paraffin. Care and use of the animals was in accordance with NIH guidelines and Institute Animal Care and Use Committee regulations. Local ethical committee permission was received for this study.

Immunocytochemistry

Five μm thick paraffin sections were removed from tissues then deparafinized and rehydrated. Immunocytochemistry was performed using a monoclonal antibody against pan-cadherin and avidin-biotin-peroxidase method (Zymed®, San Francisco CA, U.S.A.). The monoclonal anti-pan-cadherin antibody (clone CH-19), which was generated by using a synthetic peptide corresponding to the C-terminal amino acids of chicken N-cadherin with an extra N-terminal lysine residue as immunogen showing reaction with a distinct 135 kD band in a wide variety of tissue and species was from Sigma (St. Louis MO, U.S.A.). Sections were washed several times with phosphate buffered saline-triton X-100 (PBS-Tx) and they were pre-treated with 3% hydrogen peroxide for 10 min to eliminate endogenous peroxidase activity. To reduce non-specific staining, sections were pre-treated with normal rabbit serum for 20 min. Sections were then incubated with a 1/200 dilution of anti-pan-cadherin antibody for 24 h at 4°C in a humidified chamber. Following washing in PBS-Tx, biotinylated secondary antibody (Histostain plus kit, Zymed®) was applied for 15 min at room temp. Following washing in PBS-Tx, streptavidin-peroxidase conjugate (Histostain plus kit, Zymed®) was applied for 15 min at room temp. After washing in PBS-Tx, 0.6% H_2O_2 and 3,3′-diaminobenzidine (DAB) was applied as chromogen for 3–5 min at room temp. Sections were examined by light microscopy (BX5OF-3; Olympus, Tokyo, Japan). Control sections were incubated in the absence of primary antibody.

RESULTS

Pan-cadherin immunoreactivity (IR) was demonstrated in the following tissues: the secretory cells of the adenohypophysis (Fig. 1); in the myelin sheath and on Schwann cell membranes of the autonomic nerve (Fig. 2); in the basal cells of the stratified epithelial cells of the cornea (Fig. 3); in the nerve plexus of the basal layer of the stratified squamous epithelium of the oesophagus (Fig. 4); in the gastric glandular cells of the corpus of the stomach (Fig. 5); in foveolar epithelium and pyloric glandular cells of the pylorus (Fig. 6); in the basal

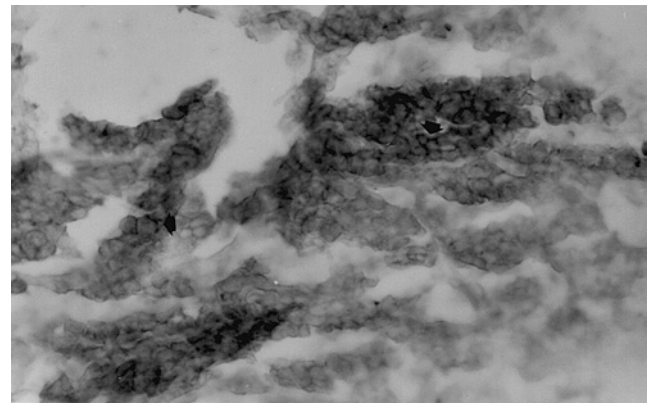


Fig. 1. Pan-cadherin immunoreactivity is present in the secretory cells (arrows) of the adenohypophysis. × 400.

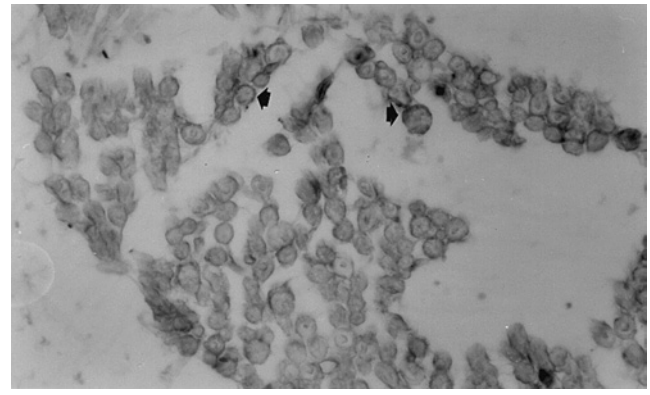


Fig. 2. Pan-cadherin immunoreactivity is present in the myelin sheath and in Schwann cell membranes, and also in axis cylinder (arrows) of the autonomic nerve. × 400.

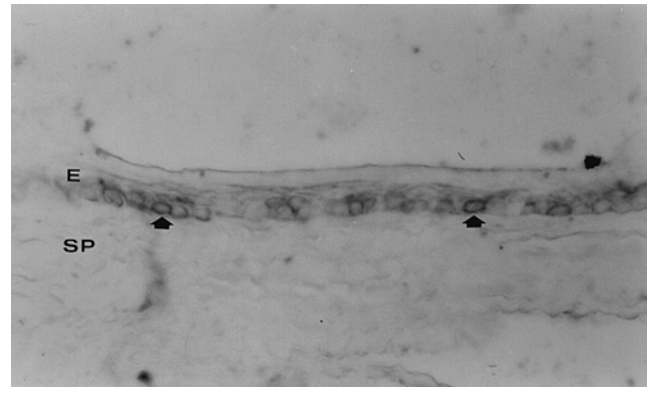


Fig. 3. Pan-cadherin immunoreactivity is dense and abundant in the basal cells (arrows) of stratified epithelium of the cornea. × 200. E: cornea epithelium, SP: substantia propria.

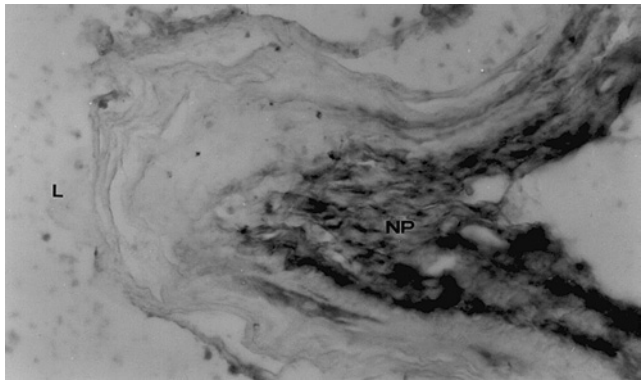


Fig. 4. Pan-cadherin immunoreactivity is positive in the nerve plexus of the basal layer of the stratified squamous epithelium of the oesophagus. × 400. L: lumen. NP: nerve plexus.

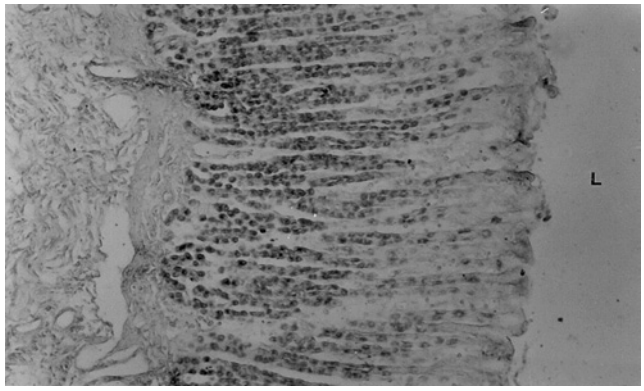


Fig. 5. Pan-cadherin immunoreactivity is intense and abundant in the gastric glandular cells of the corpus of the stomach. × 100. L: lumen.

surfaces of the villus epithelial cells and in some connective tissue cells of the duodenum and jejunum (data not shown), in the villus epithelium of the ileum (Fig. 7), and in its myenteric plexus (Fig. 8); in the alveolar cells of the alveoli, in the interalveolar septum, and in the capillary wall of the lung (Fig. 9), and also in the smooth muscle cells of the bronchi (data not shown); in the apical cytoplasm of the luminal sides and brushborder of the proximal convoluted tubules of the kidney (Fig. 10); in the islet cells of Langerhans, in the acinar cells of the exocrine pancreas, and in the vascular wall (Fig. 11). For the first time, pan-cadherin-IR was demonstrated in the pigmented and nonpigmented epithelial cells of the corpus ciliaris (Fig. 12), and in the nerve plexus around the smooth muscle cells of the corpus cavernosum of the penis (Fig. 13). Control sections did not show any immunostaining. The intensity of pan-cadherin-IR in various organs is shown in Table 1.

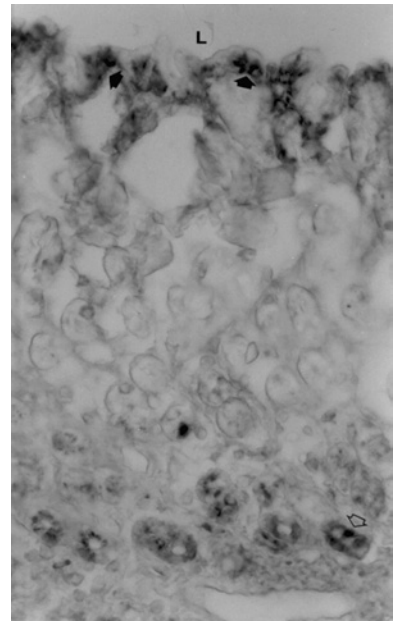


Fig. 6. Pan-cadherin immunoreactivity is positive in foveolar epithelium (solid arrows) and in pyloric glandular cells (open arrow) of the pylorus. × 200. L: lumen.

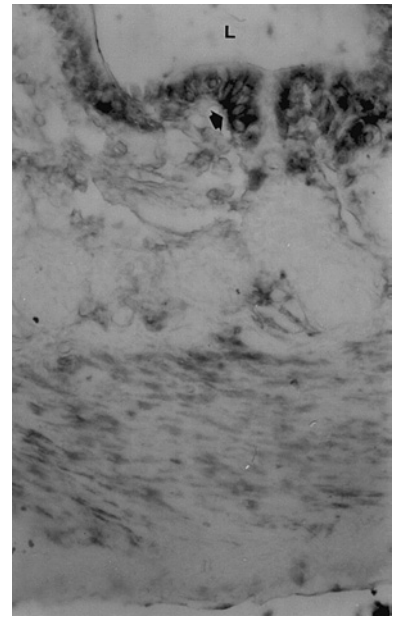


Fig. 7. Pan-cadherin immunoreactivity is present in some epithelial cells (arrow) surrounding the villi of the ileum. × 400. L: lumen.

DISCUSSION

The present study provides immunocytochemical evidence for the localization of pan-cadherin-IR in many tissues and organs of normal adult rats. The immunocytochemical localization of pan-cadherin described here is in good agreement with the

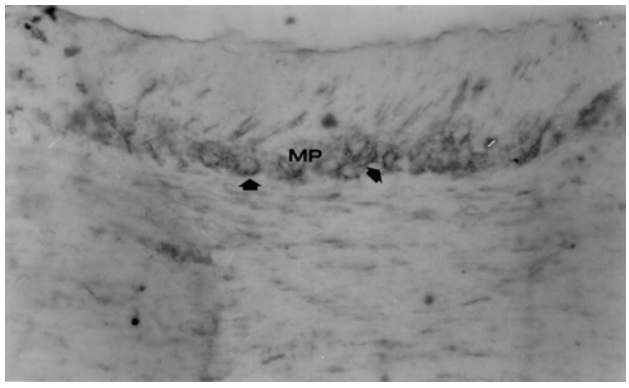


Fig. 8. Pan-cadherin immunoreactivity is positive in the nerve plexus (arrows) between the inner circular and outer longitudinal muscle cells of the ileum. × 400. MP: myenteric plexus.

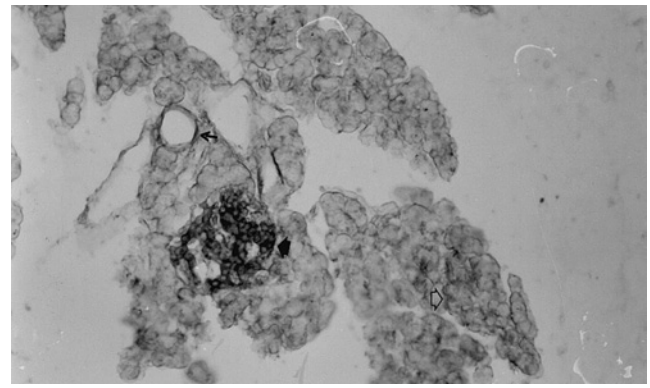


Fig. 11. Pan-cadherin immunoreactivity is dense in the islet cells of Langerhans (solid arrow), but it is weak in the acinar cells (open arrow) of the exocrine pancreas, and on the vessels (arrow). × 200.

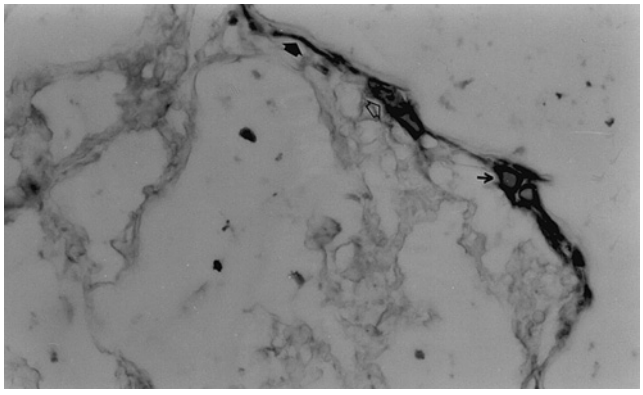


Fig. 9. Pan-cadherin immunoreactivity is dense in the alveolar cells (solid arrow) of the alveoli, in the interalveolar septum (open arrow), and in the capillary wall (arrow) of the lung. × 400.

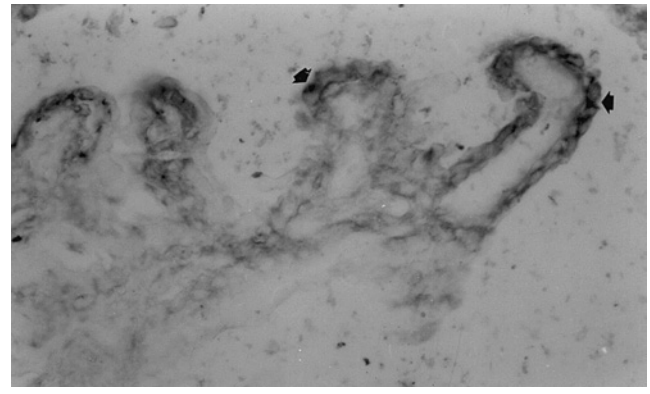


Fig. 12. Corpus ciliaris epithelial cells (arrows) showing pan-cadherin immunoreactivity. × 200.

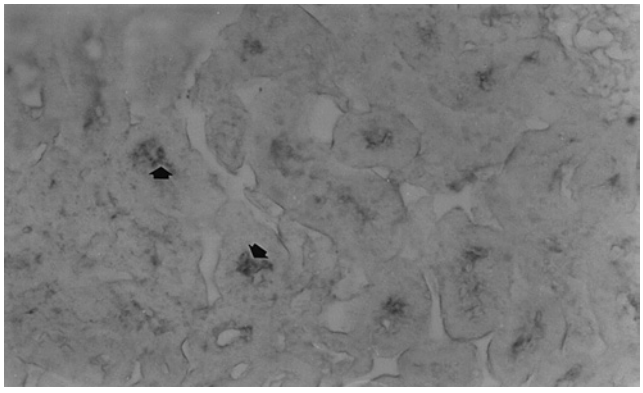


Fig. 10. Apical cytoplasm of the luminal sides and brushborder of the proximal convoluted tubules (arrows) of the kidney shows weak pan-cadherin immunoreactivity. × 400.

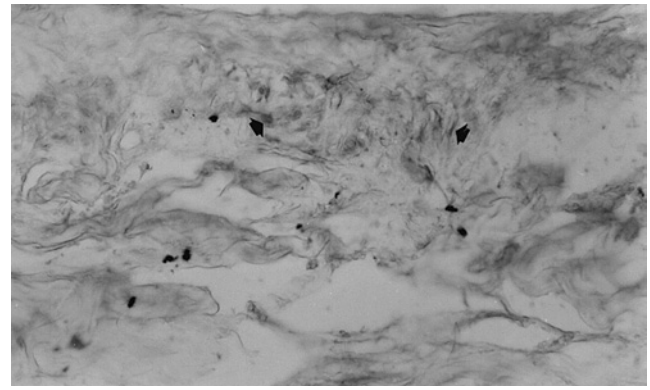


Fig. 13. Nerve bundles (arrows) around the smooth muscle cells of the corpus cavernosum of the penis show pan-cadherin immunoreactivity. × 200.

findings of several studies. For the first time, this study has determined the localization of pan-cadherin in corpus ciliaris and penis.

In the present study, we found pan-cadherin-IR on almost all secretory cells of adenohypophysis similar with the results of other study (Sugimoto *et al.*, 1996). Pan-cadherin expression pattern in the

Table 1.
Distribution of pan-cadherin immunoreactivity in adult rat tissues

Tissues	Dense	Moderate	Weak	None
Adenohypophysis				
secretory cells	+			
Autonomic nerve				
myelin sheath		+		
Schwann cells		+		
axis cylinder		+		
Cornea				
basal cells of the epithelium			+	
Oesophagus				
nerve plexus	+			
Stomach (corpus)				
glandular cells	+			
Stomach (pylorus)				
foveolar epithelium	+			
glandular cells		+		
Duodenum				
basalis of the epithelium			+	
connective tissue		+		
Jejunum				
epithelial cells		+		
Ileum				
epithelial cells		+		
myenteric plexus		+		
Lung				
alveolar cells	+			
interalveolar septum	+			
smooth muscle cells of the bronchi		+		
capillary wall	+			
Kidney				
proximal convoluted tubules			+	
Pancreas				
Langerhans islets	+			
acinar cells			+	
vascular structures			+	
Corpus ciliaris				
epithelial cells		+		
Penis (corpus cavernosum)				
nerve plexus around the smooth muscle cells			+	
Liver				+
Submandibular gland				+

autonomic nerve is identical to that described by others for the N-cadherin in the chicken sciatic nerves (Cifuentes-Diaz *et al.*, 1994; Shibuya *et al.*, 1995), and for the VE-cadherin in human peroneal nerve (Smith *et al.*, 1998). We demonstrated pan-cadherin-IR in the basal cells of the stratified squamous epithelium of the cornea, whereas it was reported in the literature before, that there was no E-cadherin-IR in normal human corneal epithelium (Scott *et al.*, 1997). Although we observed pan-cadherin-IR only in the nerve bundles present in the basal layer of the stratified squamous epithelium of the oesophagus, elsewhere, E-cadherin was reported to be present

throughout the normal human oesophageal epithelium (Dobson *et al*., 1994).

In the stomach, E-cadherin was found to be expressed strongly in normal gastric foveolar epithelium in human (Oka *et al*., 1992; Blok *et al*., 1999). Our results on the rat stomach are in general agreement with human studies. Moreover, we observed dense pan-cadherin-IR in the gastric glandular cells, pyloric glandular cells, and foveolar pyloric epithelium. Our findings on the adult rat were parallel with the previous studies in the small intestine (Sjodin *et al*., 1995; Hanby *et al*., 1996; Gessner and Tauber, 2000) and in the lung (Kasper *et al*., 1996)

N-cadherin immunoreactivity was found in the proximal convoluted tubule and E-cadherin was determined in all tubular segments except the proximal convoluted and straight tubule in the adult human kidney (Nouwen *et al*., 1993) and in the cell culture (Tani *et al*., 1995). However, we demonstrated pan-cadherin-IR only in the apical cytoplasm of the luminal sides or brushborders of the proximal convoluted tubules. According to the present study, in the pancreas, pan-cadherin-IR was dense in the islets of Langerhans, and weak in the acinar cells of the exocrine pancreas. Besides, there was positive immunoreactivity around the vascular structures. The expression of cadherin molecules in islets of Langerhans of neonatal, young and adult rats was analysed and detected that E-cadherin is the only cadherin molecule expressed in normal rat islet cells (Møller *et al*., 1992). E-cadherin was also detected on both exocrine and endocrine tissue of the pancreas of the adult rat (Cirulli *et al*., 1994).

In the present study, for the first time, pan-cadherin-IR was demonstrated in the pigmented and nonpigmented epithelial cells of the corpus ciliaris. This finding suggest that cadherins may play a role in the maintenance of normal tissue architecture and may critical for the maintenance of cell polarity. Also, for the first time, pan-cadherin-IR was demonstrated in the nerve plexus around the smooth muscle cells of the corpus cavernosum penis which suggest the role of cadherins at the neuromuscular junction.

In conclusion, our results suggest that cells in many tissues and organs of the adult rats synthesize cadherins. There is a clear link between the localization of cadherins and cell-to-cell contact. Both neural and non-neural, and secretory elements displayed cadherin immunoreactivity. The wide distribution of cadherins indicates that cadherins originates and synthesized from multiple cellular sites.

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