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Intercellular Communication and the Control of Tissue Growth: Lack of Communication between Cancer Cells

Evidence for direct cell-to-cell communication is now available for a wide variety of epithelial tissues[1–4]. At the surfaces of cell contact (junctional surfaces), the cell membranes in these tissues are normally so permeable that many cellular ions and molecules may diffuse rather freely from one cell interior to the next. This report deals with the question of whether cellular communication of this kind is involved in the control of tissue growth.

It has long been suspected that the normal growth of tissues depends on some form of contact interaction between cells. Harmonious growth requires, among other things, that cells stop moving and growing at the right place. Instructive, in this regard, is the behaviour of cells in tissue culture growing on glass surfaces. The cells stop moving and growing when they touch each other, and stop only then[5–7]. Some kind of signal must be transmitted from cell to cell on contact. The question is then whether cell-to-cell diffusion of substances is involved in the signal transmission.

A direct approach to this question seems quite hopeless until specific signal substances are identified. But one may try an indirect approach and see whether cellular communication is altered in situations of uncontrolled cellular growth. The most notorious lack of growth control occurs in cancer. Cancer cells, unlike normal ones, stop neither moving nor dividing on cellular contact, as is seen particularly clearly in cancer cells in tissue culture growing on glass surfaces[8–10]. For this reason we have undertaken to examine intercellular communication in cancer cells.

Our technique for testing intercellular communication consists essentially of passing a current of ions from inside a cell and determining what fraction of the current leaks to an adjacent cell. The technique is readily applicable to many cell systems, and provides quantitative information[1,2]. All that is required for an examination of the question is a cancer material with cells sufficiently large and stable for intracellular electrode impalement, and one the normal cell counterpart of which presents good intercellular communication. A suitable material is rat liver. The general procedure was to insert a series of microelectrodes into two adjacent cells of the surface of the liver or liver tumour in order to pass current and to record the resulting resistive membrane voltages as illustrated in the inset of Fig. 1. (The placement of current sources in both cells permitted testing of cell surface membrane integrity and of membrane sealing around the electrodes.) The ratio of membrane voltage V_{II}/V_I in the two cells (hereafter referred to as communication ratio) provides a convenient index of communication. The following types of liver cancers were used. (1) Primary cancer: induced by 3′-methyl-4-dimethylamino-azo-benzene, and transplanted cancers; (2) Morris's No. 7787; (3) Morris's No. 7793; and (4) Novikoff's. (The cancer material in order of quotation, was kindly provided by Dr. S. Sorof, Institute for Cancer Research, Philadelphia; Dr. J. Roth, University of Connecticut; and Dr. E. Hirschberg, Columbia University. We thank Dr. R. Lattes, Columbia University, for histopathological examinations of all our cancer material.)

All liver cancers lacked the intercellular communication characteristic of normal liver. Whereas normal liver cells communicate through low junctional membrane resistances and communication is detectable electrically over distances of many cells in all directions through the liver, cancer cells have no detectable communication at all (Fig. 1). Their communication ratio is less than 0·002 (the limit of resolution of our method) as compared with 0·6 in normal liver cells. This is not simply due to lower surface membrane resistance in cancer cells. Since the effective cell membrane resistance (that is, the resistance measured between cell interior and exterior) in cancer cells is actually greater than in normal cells, it is clear that, whatever the change in surface membrane resistance, the junctional resistance must be greater than in normal cells. While the junctional membranes in normal cells are several orders of magnitude more permeable than the non-junctional ones[2,3], the junctional membrane surfaces in cancer cells are at least as impermeable as the non-junctional surfaces; the effective membrane resistance in cancer cells is 20–100 times greater than in normal cells (Table 1). The behaviour of cancer cells is, in this respect, similar to that of normal cells after their intercellular communication is cut off artificially and their junctional membranes are sealed[4,11,12]. The differences in junctional

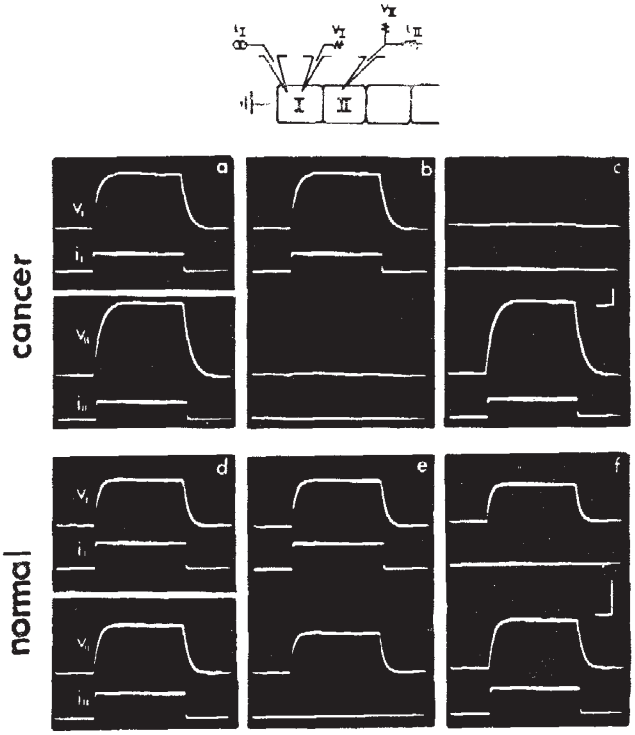

Fig. 1. Lack of communication in cancer cells (Novikoff's rat liver cancer). *a*, To test integrity of surface membranes, current ($i_I = 9 \times 10^{-9}$ amp) is passed from a microelectrode inside cell I to cell exterior (grounded), and the resulting resistive voltage (V_I) is recorded with another microelectrode in this cell (upper two oscilloscope traces). Current is then passed through the adjacent cell II ($i_{II} = 1{\cdot}8 \times 10^{-8}$ amp) and voltage recorded in cell II (lower two traces). (In cell II the same microelectrode, connected to a balanced bridge circuit, serves for both passing of current and recording of voltage.) *b*, *c*, To test intercellular communication, current is passed alternately from cell I (*b*) and from cell II (*c*), and the voltages are recorded simultaneously in the two cells. For comparison, a similar sequence is shown in *d*–*f* for normal rat liver cells ($i_I = 1{\cdot}3 \times 10^{-7}$ amp; $i_{II} = 2{\cdot}8 \times 10^{-7}$ amp). Calibration all records: voltage, 10 mV; time, 20 msec

Table 1

Liver preparation	Communication ratio V_{II}/V_I*	Effective cell resistance* 10^5 Ω	No. of cases
Normal	0·6 ± 0·01	2·57 ± 0·05	100
Primary cancer, induced by azo-dye	< 0·002	98	1
Transplanted cancers			
Morris's No. 7787	< 0·002	60 ± 7	24
Morris's No. 7793	< 0·002	243 ± 16	29
Novikoff's	< 0·002	88 ± 10	13

* Mean values with their standard errors. The differences in communication ratio and effective cell resistance between normal and cancer liver cells are, in all cases, with multiple data significant at a level better than 0·001.

communication are so marked that they offer a means for identifying cancer with ease at the cellular level (Table 1).

Of further interest is that cancer cells induce changes in communication among normal cells. This is seen most clearly during the early stages of cancer invasion, at a time when the first cancer cells (originating, for example, from a cell suspension in the peritoneal fluid) are seen to grow on the normal liver. A fringe is then often found around the cancer cells, containing cells normal by histological standards, but with significantly less communication; cells beyond this fringe present normal communication. For example, in an early phase of liver invasion by Morris's cancer cells No. 7793, the communication ratio in the fringe was 0·3, and the effective cell resistance, 12×10^5 Ω, both differing from normal values at a level of significance better than 0·001 (see Table 1). This ability to induce changes in communication and, perhaps, eventually even disconnexion in normal cells was found in the fast-growing cancers, such as Novikoff's, as well as in the slowly growing ones, such as Morris's No. 7787.

In conclusion, the surface membrane of the liver cancer cell is a strong barrier to diffusion which, unlike that of the normal cell, is continuous all around the cell, including the surfaces of cell contact. Cancer cells are thus unable to engage in the kind of communication possible in their normal counterparts.

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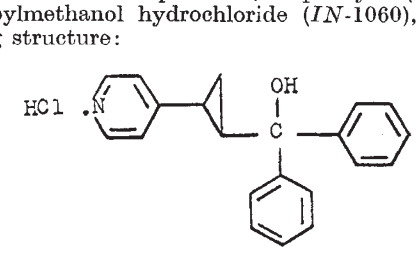
PHARMACOLOGY

Pharmacological Investigation of a New Psychoactive Agent

Parallel preclinical and clinical investigations were performed on the compound α,α-diphenyl-2-(4-pyridyl)-*cyclo*propylmethanol hydrochloride (*IN*-1060), with the following structure:



IN-1060

Neither the chemical structure nor the reported pharmacological action of this compound was typical of the iminodibenzyl- or phenothiazine-type psychotherapeutic agents. The results of the clinical investigation are reported elsewhere[1]. This compound was investigated because it significantly altered aggressive behaviour[2] and drug-induced aberrant behavioural states[3] in dogs, and yet it did not produce any apparent effects on normal animal behaviour[2].

Five conscious dogs with surgically prepared exteriorized carotid arteries in skin loops were used throughout. In this experimental preparation, using techniques previously described[4], we recorded the arterial pressure responses produced by intravenously injected *IN*-1060, and its effects on the responses produced by fixed intravenous doses of autonomimetic agents. These challenging agents and the doses used were: adrenaline (Adr), 2 μg/kg; acetylcholine (ACh), 0·6 μg/kg; noradrenaline (NAd), 2 μg/kg; histamine (Hist), 1 μg/kg; tyramine (Tyr), 0·2 mg/kg; 5-hydroxytryptamine (5HT), 10 μg/kg; and yohimbine (Yoh), 0·5 mg/kg. Each compound was administered at least 10 min apart and after the arterial pressure returned to basal level following treatment with *IN*-1060. Control responses to the challenging drugs are expressed as the average of three determinations obtained previously in each dog.

The results of this experiment are summarized in Table 1. It was found that the intravenous administration of 10 mg/kg of *IN*-1060 produced an average increase in calculated mean arterial pressure of 91·8 mm mercury. However, in studies at present in progress, oral doses as high as 30 mg/kg/day, for 7 consecutive days, did not alter existing normotensive arterial pressure levels.

Table 1. Effect of *IN*-1060 on Mean Arterial Pressure and Responses to Autonomimetic Drugs in Conscious Dogs

	IN-1060	Tyr	ACh	Adr	Hist	NAd	5HT	Yoh
Average control response	—	+46	−33	+41	−18	+39	+29	+63
Average response after *IN*-1060	+91·8	+56	−22	+53	−12	+54	+26	+61
P value*	—	< 0·10	< 0·05	0·20	0·20	0·025	—	—

* *P*, Value obtained by paired comparison '*t*' test.

The responses of some challenging drugs, when compared with the control values, were significantly altered by the *IN*-1060 treatment. It was observed that the treatment augmented the pressor responses to tyramine, adrenaline and noradrenaline, inhibited the depressor responses to acetylcholine and histamine, but had no apparent effect in the pressor responses produced by 5-hydroxytryptamine and yohimbine.

It is of interest to compare these results with the reported actions of imipramine and chlorpromazine. The potentiation of the pressor responses to noradrenaline and the inhibition of the depressor responses to acetylcholine and histamine after *IN*-1060 are similar to the actions reported for imipramine[5]. However, the augmented adrenaline and tyramine responses after *IN*-1060 are contrary to the findings reported for imipramine[5,6]. Intravenous treatment with chlorpromazine resulted in a depression of the arterial pressure with associated effects of reversal of the pressor response produced by adrenaline, and inhibition of the pressor responses to tyramine, noradrenaline and yohimbine[7]. The finding that *IN*-1060 treatment did not alter the arterial pressure responses to 5-hydroxytryptamine or yohimbine also deviates from the reports on the effects of acute treatment with imipramine[8] and chlorpromazine[7]. The pressor response to 5-hydroxytryptamine is inhibited by acute intravenous administration of imipramine[9] and chlorpromazine[7]. The pressor response to 5-hydroxytryptamine is inhibited by acute intravenous administration of imipramine[9] and chlorpromazine[7]. The pressor response of yohimbine is inhibited by acute intravenous chlorpromazine[7,10], but potentiated by imipramine[8].

It is concluded that the effects observed are not typical of chlorpromazine or imipramine actions. This confirms