Biochimica et Biophysica Acta, 776 (1984) 337–339
Elsevier

BBA Report

BBA 70182

THE INSECTICIDE DDT DECREASES MEMBRANE POTENTIAL AND CELL INPUT RESISTANCE OF CULTURED HUMAN LIVER CELLS

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(Received April 24th, 1984)

Key words: Membrane potential; Cell input resistance; Insecticide; DDT; Cell culture; (Liver)

The resting membrane potential, E_m, and the cell input resistance, R_{inp}, of cultured human Chang liver cells were measured using the single electrode 'double-pulse' current clamp technique, following exposure of the cells to the insecticide DDT (20 μM). In control (unexposed) cells, the mean E_m was −24 mV, and the mean R_{inp} was 30 MΩ. Neither parameter was significantly impaired after 1 h of cell exposure to DDT. But after 7 and 48 h, the E_m was depolarized by 15 and 25 mV, respectively, in parallel with a decrease of the cell input resistance. The strongly time-delayed effect of DDT on Chang liver cell membranes may indicate a mode of interaction different from excitable membranes.

Knowledge of reaction mechanisms of pesticides in biological cells is indispensible for risk evaluation of potential hazards in animals and man. We have been studying the interaction of pesticides with mammalian cell plasma membranes of cultured Chang liver cells, an established cell line of human origin. The insecticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) was shown to interact with plasma membrane proteins [1,2]. Endogenous tryptophan fluorescence of membrane proteins was decreased [2], indicating interference of the insecticide with membrane proteins, and binding of the hormone antagonist hydroxybenzylpindolol to the β-adrenergic receptor was diminished [1], indicating a specific loss in the apparent number of β-receptors.

The effect of DDT on these membrane proteins was not parallel in time with the uptake of the insecticide by the cells: whereas the physical process of uptake into the cell plasma membrane came to equilibrium within 6 h of cell exposure to DDT, it was 48 h before either biological effect was significantly developed [2]. On the other hand, the activity of some other plasma membrane enzymes was not affected under identical experimental conditions [2]. Therefore, we set out to define closer the degree of specificity of DDT interaction with these cells.

Electrophysiological parameters, such as the resting membrane potential [3] and the cell input resistance (terminology as in Ref. 4), appear to reflect cellular damage sensitively. In order to determine the response of these membrane parameters under the conditions of cell exposure to the lipophilic insecticide DDT, we applied the 'double-pulse' current clamp technique (Refs. 5, 6 and legend to Fig. 1) on cultured human liver cells. We took advantage of the following characteristics of this method: (i) reduction of leakage by single electrode impalements only; (ii) potential-controlled audio signal indication of correct electrode insertions (in addition to optical control); (iii) sampling of signal amplitudes in the voltage re-

Abbreviation: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane.

laxation, thus avoiding overshoot phenomena of electrode resistance; (iv) polarity change for every 'double-pulse', thus preventing polarization artefacts on the membrane potential.

Chang liver cells (ATCC No. CCL 13) were cultured in monolayer as described earlier [2]. About $2 \cdot 10^6$ cells were plated per culture dish (Falcon, 10 cm in diameter) on the day preceding the experiment. Cell density was sufficient to provide proper physical contact between cells in order to establish homogenous electrical coupling between them. Fig. 1. shows typical chart records of the membrane potential E_m and the cell input resistance R_{inp}. Under steady-state conditions, i.e., after 4–6 min of electrode impalements, both parameters were clearly different from controls in cells which had been exposed to DDT for 7 and 48 h. Table I compiles mean values and standard deviations of E_m and R_{inp} in experiments conducted after 1, 7, and 48 h of cell exposure to DDT, together with an evaluation of their statistical significance. As shown in the table, exposure of cells to DDT for 1 h has an insignificant effect on both parameters. In contrast, exposure for 7 and 48 h depolarized the membrane potential in the mean by 15 and 25 mV, respectively, in parallel to a significant decrease of the cell input resistance. The mean membrane potential of Chang liver cells compares favorably with values reported for other membrane potentials in monolayer cultured mammalian cells [7,8]. It is noteworthy that the membrane potential of control cells appears to increase

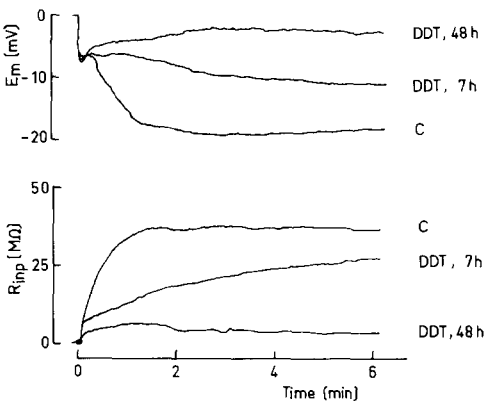

Fig. 1. Electrophysiological measurement of the membrane potential, E_m, and the cell input resistance, R_{inp}, of Chang liver cells. Typical simultaneous recordings with time of E_m (upper panel) and of R_{inp} (lower panel, positive current) in three cells are shown. Cells were impaled at time zero following no (C, control), 7 h (DDT, 7 h), and 48 h (DDT, 48 h) exposure to DDT (20 μM, cf. Ref. 1). The basics of the electrophysiological measurements are described elsewhere [5]. Briefly, cells were impaled by a single-barreled microelectrode which served both for potential recording and current injection. Bipolar 'double-pulse' current stimuli were injected into the cell and signal amplitudes were sampled in the voltage response of the circuit at distinct times carefully adjusted to the different time constant of the electrode (τ_e) and of the cell membrane (τ_{inp}), respectively ($\tau_{inp}/\tau_i > 50$). Values of E_m, R_{inp} and of the electrode resistance (not shown here) were calculated by differential amplifiers and were recorded with a y, t multi-pen recorder. The current-voltage relationship was nearly linear for voltage deflections smaller than 50 mV from the resting potential. Adjustments for the Electrophysiological Monitor ELM2 were [4]: short pulse, 1 ms; long pulse, 100 ms; t (SH1) = 200–300 μs; $I = \pm 1$ nA. Electrode resistances were from 40–60 MΩ and tip potentials were smaller than −3 mV in the experimental solution.

TABLE I

MEMBRANE POTENTIAL AND CELL INPUT RESISTANCE OF CHANGE LIVER CELLS

Cells were exposed to growth medium without DDT (control) or to growth medium containing 20 μM DDT (+DDT). Impalements were started after the exposure times indicated, and the electrophysiological experiments were performed within 1 h. Two or three dishes of each preparation were used. E_m and R_{inp} data are given as the mean value ± S.D. (number of impalements). Statistics: Expt. 1, E_m: $P \leqslant 0.2$; R_{inp}: $P \leqslant 0.5$; both not significantly different from control (marked *). Expt. 2, E_m: $P \leqslant 0.002$; R_{inp}: $P \leqslant 0.02$. Expt. 3, E_m: $P \leqslant 0.001$; R_{inp}: $P \leqslant 0.001$; all values significantly different from controls (t-test).

Expt.	Exposure	Exposure time (h)	E_m (mV)	R_{inp} (MΩ)	ΔE_m (mV)	ΔR_{inp} (MΩ)
1	control	1	−18 ± 9 (6)	34 ± 14 (6)		
	+DDT	1	−12 ± 6 (8)	40 ± 15 (8)	6 *	+6 *
2	control	7	−22 ± 8 (6)	45 ± 15 (6)		
	+DDT	7	−7 ± 4 (6)	25 ± 7 (6)	15	−20
3	control	48	−28 ± 13 (6)	40 ± 12 (6)		
	+DDT	48	−3 ± 2 (5)	4 ± 3 (5)	25	−36

with the time elapsed from the last change of the growth medium (Table I), as has likewise been observed in rat hepatocytes [9].

Under our experimental conditions of cell exposure, the decrease of the electrophysiological membrane parameters E_m and R_{inp} precedes the effect of DDT on endogenous tryptophan fluorescence as well as on the β-receptor in the same membrane. On the other hand, membrane potential and cell input resistance reflect the presence of DDT rather late when compared with the time-course of DDT action on excitable nerve cell membranes. For example, an increased negative after potential as well as a decreased resting membrane potential were observed within 20 min of exposure to DDT of lobster nerve preparations [10]. When directly applied onto the outer hair wall of the moth *Antheraea polyphemus*, a complete break down of the membrane potential of the receptor cell dendrite was observed even within a few seconds [11]. One may conclude that the decrease of E_m and R_{inp} caused by DDT in Chang liver cells does not follow the mechanism of DDT poisoning in excitable cells. The action on excitable membranes is obviously dependent on the rate of access of the insecticide to the target membrane site. In contrast, the strongly delayed effects on various plasma membrane functions of non-excitable human liver cells can not be explained solely be a low rate of insecticide diffusion, but may rather indicate interaction either with separate protein targets in the cell plasma membrane or with a common target site distant from the membrane. In this way, the insecticide DDT could interfere with the metabolism of specific membrane proteins elsewhere within the Chang liver cell.

We thank Miss Eva-Maria Linderer for the preparation of the microelectrodes.

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