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PCP AND ANALOGS PREVENT THE PROLIFERATIVE RESPONSE OF T LYMPHOCYTES BY LOWERING IL2 PRODUCTION

AN EFFECT RELATED TO THE BLOCKADE OF MITOGEN-TRIGGERED ENHANCEMENT OF FREE CYTOSOLIC CALCIUM CONCENTRATION

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Abstract—The psychotomimetic drug PCP displays a vast array of known pharmacological effects, among them its capacity to affect cation transport in nervous and myocardiac tissues. Since increased movements of cations are essential for the immune responses, it has been mentioned that PCP could also depress immune functions by this mechanism. In order to check this hypothesis, we have investigated the effects of PCP and of many other structural derivatives on the blastogenic response of murine or human T lymphocytes. We find that all the drugs block an early event of T lymphocyte activation and prevent their further proliferation; conversely they do not affect primed lymphocytes. The compounds, which do not inhibit interleukin-1 (IL-1) production in stimulated macrophages, lower interleukin-2 (IL-2) synthesis in activated T helper cells. This negative action appears to be related to the inhibition of the rise of free cytosolic calcium concentration $[Ca^{2+}]_i$ observed soon after the T receptor triggering and which is an essential message for IL-2 production. The lymphocyte membrane depolarization induced by the drugs could explain the blockade of the lectin-induced $[Ca^{2+}]_i$ changes. The study of the structure–activity relationship shows that the PCP analogs which possess a quasi-rigid conformational structure express an inhibitory capacity of T lymphocyte proliferation higher than that of PCP (200 times for some products). Since these compounds interact poorly with the CNS tissues and have few behavioral effects, we suggest that PCP exerts its negative action on lymphocytes on cell components different from its receptor(s) in the CNS.

Phencyclidine (PCP) is a psychotomimetic drug that displays a vast array of pharmacological effects. Its actions on central and peripheral nervous systems in animals and in man are well documented [1–3] but its effects on the immune response have been poorly documented. A brief report has nevertheless mentioned that humoral and cellular responses were depressed in lymphocytes treated *in vitro* by PCP [4]. A study of the mechanism by which the drug affects lymphocyte functions appears important since administration or consumption of PCP may produce a schizophrenia-like syndrome [5] and since in some instances schizophrenia is considered as a CNS autoimmune disease [6]. Moreover a PCP congener, ketamine, is often used in general anesthesia during surgery and could influence postoperative infections, if it possesses immunosuppressive properties.

Activation of T lymphocytes by specific antigens (or mitogenic lectins) initiates a sequence of events which begins with the binding of the mitogen and culminates in DNA synthesis and cell proliferation. It is well established that most of the events are controlled by ion transport mechanisms and that early increases of cation fluxes are necessary steps for triggering further DNA synthesis [7–9]. Since PCP is able to modify cation movements in other tissues [10–12], it might influence lymphocyte response through this mechanism.

We have investigated the effects of PCP and some of its analogs on the blastogenic response of murine or human T lymphocytes. We report here that PCP and its congeners block an early step of lymphocyte activation and prevent lectin-induced proliferation. The inhibition efficiency depends on the molecular conformation and on the nature of the substituents of the products. We found that in fact these compounds lowered interleukin-2 (IL-2) production, an essential step in lymphocyte proliferation. This effect was consequent to an inhibition of the rise of cytosolic free calcium concentration ($[Ca^{2+}]_i$), observed after T receptor triggering, that was probably due to the membrane depolarization induced by PCP and its analogs.

MATERIALS AND METHODS

Materials. Lymphoprep was purchased from Eurobio, concanavalin A (Con A) from Pharmacia, phytohemagglutinin (PHA), RPMI 1640 from GIBCO fetal calf serum (FCS) from Boehringer, [6^3H]-thy-

midine ([^{3}H]-dThd) from CEA, Quin 2 and Quin 2 acetoxymethylester (Quin-2-AM), phorbol myristate acetate (PMA), valinomycin and A23187 calcium ionophore from Sigma, *E. coli* lipopolysaccharide (LPS) from DIFCO, 11'333434-hexamethylindodicarbocyanine iodide (DiI-C_1-(5)) from Molecular Probes.

Chemistry. Ketamine hydrochloride was a gift of Dr G. Trouiller, Vert-le-Petit, France. Compounds GK_0 (TCP), GK_1 (PCP), GK_3, GK_4, GK_6, GK_{18}, GK_{67}, GK_{117} have been synthesized and their physicochemical properties published [13, 14]. The details of the synthesis and physicochemical properties of the new PCP derivatives will be published elsewhere as well as the relationship between their structures and their biological activities.

Cells. Human mononuclear cells (HMC) were obtained by separation of blood cells from healthy volunteer donors on lymphoprep [15]. Murine thymocytes and splenocytes from Swiss mice were prepared as previously [16]. The IL-2-dependent line of murine cytotoxic T cells (CTLL) and the JURKAT cell line were a generous gift from Dr Dupuy d'Angeac (INSERM U291, Montpellier).

DNA synthesis in mitogen stimulated lymphocytes. [^{3}H]-dThd incorporation was determined in lymphoid cells stimulated with different mitogens: 10 μg/ml Con A or 5 μg/ml PHA for HMC, 2.5 μg/ml Con A or 5 μg/ml PHA or 7 ng/ml PMA + 5 μg/ml PHA or 7 ng/ml PMA + 50 ng/ml A23187 for murine splenocytes, 2.5 μg/ml Con A or 7 ng/ml PMA + 50 ng/ml A23187 or 7 ng/ml PMA + 5 μg/ml PHA for murine thymocytes, in the presence or in the absence of drugs. Cells were distributed into microtitration plates (200 μl per well) at 2×10^6 cells/ml RPMI 1640, 5% FCS, and incubated for 72 hr (HMC) or 48 hr (murine cells) at 37°. 4 hr before harvesting the cells 0.5–1 μCi [^{3}H]-dThd was added to each well and the [^{3}H]-dThd incorporated into the cells determined as previously reported [15].

Determination of interleukin-1 production in the supernatants of murine macrophages stimulated by LPS in the presence or absence of drugs. Macrophages obtained from peritoneal exudates of Swiss mice were incubated in 2.5 ml plastic dishes for 6 hr at 37° at 10 cells/ml RPMI 1640. The non-adherent cells were then washed thrice with RPMI 1640 and the adherent population supplemented with the initial volume of RPMI 1640. These cells were then cultured with or without 20 μg/ml LPS in the presence or absence of the indicated concentrations of PCP analogs at 37°. The supernatants were collected at 24 hr, centrifuged and extensively dialysed. The IL-1 activity of each supernatant was assayed by the thymocyte proliferation assay exactly as described in [17]. The response of CBA mice thymocytes (10^7 cells/ml RPMI 1640, 5% FCS, 50 μM mercaptoethanol) to 5 μg/ml PHA was studied as mentioned above in the presence of dilutions 2, 4, 8 of the macrophage supernatants [17].

Determination of IL-2 production in the supernatants of Con A stimulated splenocytes or of PMA + PHA stimulated JURKAT cells. Splenocytes ($2.5–3 \times 10^6$ cells/ml) were cultured for 24 hr at 37° in RPMI 1640, 5% FCS, in the presence of Con A (5 μg/ml) and different additives. The cells were then centrifuged, and supernatants were dialysed and their IL-2 activity was measured in a bioassay with a murine IL-2-dependent cytotoxic T cell line [9].

JURKAT cells (10^6 cells/ml RPMI 1640, 5% FCS) were stimulated to produce IL-2 with 10 μg/ml PHA + 7 ng/ml PMA for 24 hr at 37° as described in [9]. In some experiments drugs were present. The IL-2 activity of the supernatants was determined after dialysis.

At concentrations inhibiting lymphocyte blastogenesis, PCP or its analogs do not affect the response of CTLL cells to exogeneous IL-2 (data not shown), which established that IL-2 determination could not be perturbated if drug traces remained in the cell supernatants after dialysis.

Determination of $[Ca^{2+}]_i$. Experiments were performed exactly as described in [18], in 10 mM Hepes buffer, pH 7.5, 5.6 mM Na_2HPO_4, 103 mM NaCl, 5.35 mM KCl, 0.41 mM $MgSO_4$, 0.42 mM $CaCl_2$, 11 mM glucose. Thymocytes (2×10^7 cells/ml) were loaded with 40 μM Quin-2-AM and, after washing, the fluorescence of the cellular suspension (10^7 cells/ml) was determined (λ_{ex} 339 nm, λ_{em} 492 nm) with a Perkin-Elmer Hitachi spectrofluorimeter, before and after addition of 5 μg/ml Con A. $[Ca^{2+}]_i$ was calculated following the method of Rink *et al.* [19]. In some experiments the drugs, at indicated concentrations, were added to the cell suspension before or after Con A.

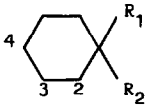
Determination of membrane potentials. Membrane potentials were determined with the fluorescent probe DiI-C_1-(5) (0.5 μM final concentration) by the method of Rink *et al.* [20], using 5×10^6 thymocytes per ml 10 mM NaHepes buffer, pH 7.2, 1 mM Na_2HPO_4, 139 mM NaCl, 6 mM KCl, 125 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5.6 mM dextrose. The fluorescent signal of the permeant product was monitored at 37° with continuous stirring (λ_{ex} 620 nm, λ_{em} 660 nm). The membrane potential was evaluated using the null point method [20] after addition of 1 μM valinomycin to the cell suspension.

RESULTS

1. *Analogues and derivatives of the PCP molecule used (Table 1)*

As the PCP molecule is known to adopt in solution a preferred conformation with an axial phenyl group and as the preferred conformation is proved to play a role in the biological activity throughout the series [13], the choice of the structures to be tested was based upon the conformational characteristics of the structures. Thus the first set of molecules was constituted by the most mobile conformations, i.e. cyclohexyl unsubstituted molecules (PCP, TCP, GK_{13}). The second set of molecules was constituted by moderately modified conformations by means of cyclohexyl substitutions (GK_4: a more equatorial phenyl group than in PCP, GK_6: a more axial phenyl group than in PCP; GK_{18}: relatively similar conformation to that of PCP). The third set of molecules was constituted by quasi-rigid conformations resulting from the substitution of an equatorial *t*-butyl group in the cyclohexyl ring, restraining the phenyl group in an axial position (GK_3, GK_{67}, GK_{117}).

Table 1. Studied PCP congeners



Code	R_1	R_2	Cyclohexyl*
GK_0 (TCP)	2-Thienyl	Piperidine	—
GK_1 (PCP)	Phenyl	Piperidine	—
GK_3	Phenyl	Piperidine	*trans*-4-t-butyl
GK_4	Phenyl	Piperidine	*cis*-4-methyl
GK_6	Phenyl	Piperidine	*cis*-3-methyl
GK_{13}	2-Benzothienyl	Piperidine	—
GK_{18}	Phenyl	Piperidine	4,4-dimethyl
GK_{67}	2-Thienyl	Piperidine	*trans*-4-t-butyl
GK_{117}	Phenyl	Dimethylamine	*trans*-4-t-butyl
GK_{138}	*m*-CH_3-phenyl	Piperidine	*trans*-4-t-butyl
GK_{144}	*p*-CH_3-methyl	Piperidine	*trans*-4-t-butyl
GK_{145}	*m*-CH_3O-phenyl	Piperidine	*trans*-4-t-butyl
GK_{154}	*p*-F-phenyl	Piperidine	*trans*-4-t-butyl
GK_{155}	*m*-F-phenyl	Piperidine	*trans*-4-t-butyl

* *cis* and *trans* refer to the spatial relationship between the substituent and R_2.

Finally the last set of structures included derivatives of GK_3 bearing electron donating (GK_{138}, GK_{144}, GK_{145}) or electron withdrawing (GK_{154}, GK_{155}) aromatic substitutions to influence the basicity of the tertiary amino group.

In addition ketamine presented a particular interest as this PCP related molecule is used as a human anesthetic drug whose preferred conformation is reversed compared to that of PCP [21].

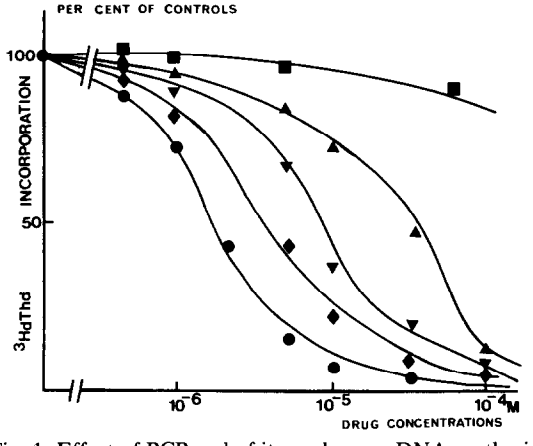


Fig. 1. Effect of PCP and of its analogs on DNA synthesis by Con A stimulated murine splenocytes. Cells were stimulated by 2.5 μg/ml Con A in the presence of different concentrations of PCP (▲), GK_3 (●), GK_4 (▼), GK_{67} (◆), or ketamine (■), and pulsed with [^{3}H]-dThd during the last 4 hr. Ordinates represent the percentage of [^{3}H]-dThd incorporated above a control value determined in the absence of drug. Measurements were performed in quadruplicates: standard deviation were lower than 10%. Control values (cpm ± SD) were 8250 ± 1200 and 324,000 ± 22,850 for unstimulated and Con A stimulated cells, respectively.

2. *Inhibitory effect of PCP or PCP congeners on T lymphocyte proliferation*

Figure 1 shows the dose-dependent inhibitory effect of PCP and of PCP analogs GK_3, GK_4, GK_{67} and ketamine on DNA synthesis in Con A (or PHA) activated murine splenocytes. Similar effects were observed with both mitogens when the drugs were added to the cell cultures at the initiation of activation. GK_3 was found a better inhibitor than PCP: it led to 50% inhibition of mitogenesis at 2.4 μM while 20-fold higher PCP concentrations were necessary to reach the same effect which was also observed with 5 μM GK_{67} and 12 μM GK_4; ketamine has no inhibitory action.

Other PCP congeners have been tested: except ketamine all of them suppressed lymphocyte blastogenesis in a similar way. Table 2 reports the drug concentrations promoting 50% inhibition of DNA synthesis in Con A activated murine splenocytes, murine thymocytes or human HMC. All drugs were more inhibitory than PCP, the most efficient being GK_{144} (200-fold better than PCP). Their capacity to suppress proliferation was similar for all lymphocyte populations. At concentrations 3–4-fold higher than those inducing 50% inhibition the drugs were not cytotoxic as assessed by Trypan blue exclusion, which explains why lymphocytes could be stimulated again after 24 hr incubation with the drugs and extensive washing.

The reversible suppressive effect of PCP or its derivatives was maximal only if the drugs were added during the first 15 hr following activation; it was abrogated when they were added during the last 24 hr of the culture (data not shown).

3. *PCP and analogs do not inhibit macrophage IL-1 production but prevent IL-2 formation by helper T cells*

It is now accepted [22] that the mitogenic effects of lectins on T cell necessitates lymphokine pro-

Table 2. Inhibitory effect of PCP and of its congeners on mitogen-induced T lymphocyte proliferation

Drug added	Murine splenocytes	Murine thymocytes	HMC
GK	38.0 ± 12.0	30.0 ± 18.0	30.0 ± 10.0
GK_1	45.0 ± 11.0	48.0 ± 10.0	54.0 ± 10.0
GK_3	2.40 ± 1.20	2.50 ± 1.10	2.00 ± 1.00
GK_4	12.0 ± 1.20	12.5 ± 5.00	15.0 ± 5.00
GK_6	10.0 ± 6.00	13.0 ± 4.00	20.0 ± 5.00
GK_{13}	10.0 ± 2.00	8.00 ± 3.00	9.00 ± 3.00
GK_{18}	8.60 ± 3.50	8.80 ± 2.50	6.00 ± 1.00
GK_{67}	5.00 ± 1.00	4.30 ± 1.50	5.00 ± 2.00
GK_{117}	n.d.	5.00 ± 1.00	n.d.
GK_{138}	0.76 ± 0.12	0.76 ± 0.21	n.d.
GK_{144}	0.26 ± 0.15	0.60 ± 0.20	0.20 ± 0.10
GK_{145}	0.70 ± 0.10	1.00 ± 0.50	0.60 ± 0.20
GK_{154}	0.88 ± 0.15	1.10 ± 0.30	n.d.
GK_{155}	1.30 ± 0.50	1.20 ± 0.50	n.d.
Ketamine	n.d.	>100	>100

Drug concentrations (μM) promoting half inhibition of DNA synthesis in Con A activated murine splenocytes or murine thymocytes or HMC. Experimental conditions were those of Fig. 1; a mean of three different determinations is presented.

duction for the culmination of either cellular or humoral immune responses. Interleukine-1 (IL-1) and interleukine-2 (IL-2) are synthesized by accessory cells (macrophages) and helper T cells respectively soon after stimulation by antigen (or lectin). Because the inhibition of lymphocyte proliferation by PCP and analogs results from the blockade of an early stimulation step, we have measured the effects of these drugs on IL-1 or IL-2 production.

We found that PCP and its derivatives did not affect IL-1 production from LPS stimulated murine macrophages, even at concentrations double those required for inhibition of lymphocyte blastogenesis; on the other hand, they suppressed IL-2 accumulation in the supernatants of Con A stimulated murine splenocytes in a dose-dependent manner (data not shown). At all concentrations, the blockade of IL-2 production paralleled the inhibition of lymphocyte proliferation, the doses producing 50% inhibition of both effects being similar (Table 3). Similar PCP and its derivatives inhibited IL-2 production by stimulated JURKAT cells (Table 3). This human leukemia T cell line is often used as a model for T cell activation and can be activated in absence of accessory cells by PMA and PHA to produce IL-2.

4. *Effect of PCP or GK_3 on T lymphocyte proliferation induced by the combination of PMA and the calcium ionophore A23187*

The activation of T lymphocytes by antigens or mitogenic lectins proceeds via the inositol lipid pathway [23, 24] which delivers two necessary signals: rise in cytosolic free calcium concentration $[Ca^{2+}]_i$ and protein kinase C activation. The requirement of these signals can be bypassed by a combination of a calcium ionophore A23187 and PMA that is a protein kinase C activator [25, 26].

Figure 2 shows that PCP and GK_3 differently affected the proliferation of murine thymocytes or splenocytes induced by Con A or by PHA + PMA or by PMA + A23187: that induced by the calcium ionophore and PMA was slightly (splenocyte) or not (thymocytes) affected by drug concentrations which prevented the mitogenesis induced by Con A. Similar data, obtained with the other drugs, suggest that the lectin induced event bypassed by the ionophore is the target of the drug action: this $[Ca^{2+}]_i$ rise triggered by the lectin binding is probably affected by PCP and its derivatives.

5. *Inhibitory effects of PCP and analogs on Con A induced $[Ca^{2+}]_i$ rise in lymphocytes*

Con A triggers a rapid change in thymocyte $[Ca^{2+}]_i$ which increases more than twice (from 110 nM to 240 nM) [18]. Using the fluorescent probe Quin-2 we have monitored the thymocyte $[Ca^{2+}]_i$ increase (Fig.

Table 3. Inhibitory effect of PCP and its congeners on IL-2 synthesis by stimulated murine splenocytes or by stimulated JURKAT cells

Drug added	Murine splenocytes	JURKAT cell line
GK_0	46.0 ± 12.0	56.0 ± 20.0
GK_1	43.0 ± 18.0	45.0 ± 10.0
GK_3	2.60 ± 0.50	1.80 ± 1.50
GK_4	10.0 ± 2.00	n.d.
GK_6	11.0 ± 5.00	17.0 ± 7.00
GK_{13}	12.5 ± 2.50	n.d.
GK_{18}	6.40 ± 3.10	n.d.
GK_{67}	5.00 ± 2.00	10.0 ± 1.00
GK_{138}	0.75 ± 0.15	n.d.
GK_{144}	0.55 ± 0.25	n.d.
GK_{145}	0.95 ± 0.35	n.d.
GK_{154}	1.00 ± 0.50	n.d.
GK_{155}	1.20 ± 0.70	n.d.
Ketamine	>100	>200

Drug concentrations (μM) promoting half inhibition of IL-2 synthesis by Con A stimulated murine splenocytes or by the human T cell line JURKAT stimulated by PHA + PMA. Mean of three different experiments.

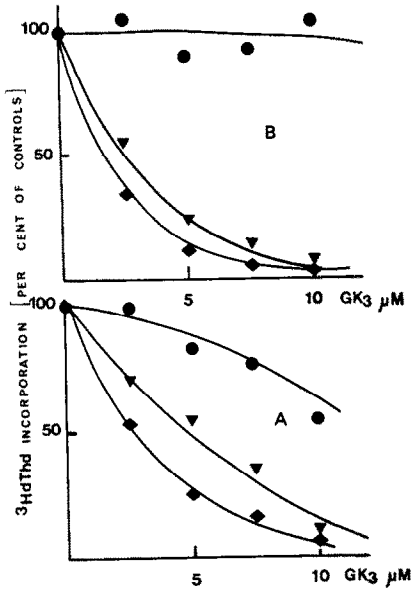


Fig. 2. Effect of GK_3 on DNA synthesis by murine thymocytes of splenocytes stimulated by different mitogens. Cells were stimulated for 48 hr with 2.5 μg/ml Con A (◆) or 10 μg/ml PHA + 7 ng/ml PMA (▼) or 50 ng/ml A23187 + 7 ng/ml PMA (●) in the presence of GK_3 concentrations. [^{3}H]-dThd incorporation (cpm) was determined as in Fig. 1. Ordinates are the percentages of [^{3}H]-dThd incorporated above controls in the absence of GK_3. Part A: splenocytes; control values were 10,850 ± 1,320, 235,372 ± 22,000, 225,000 ± 12,500, 462,350 ± 26,700, for unstimulated, Con A, PMA + PHA, PMA + A23187 stimulated cells, respectively. Part B: thymocytes; control values were 1800 ± 200, 374,820 ± 14,800, 236,500 ± 27,200, 225,700 ± 23,200, for unstimulated cells, Con A, PMA + PHA, PMA + A23187 stimulated cells, respectively.

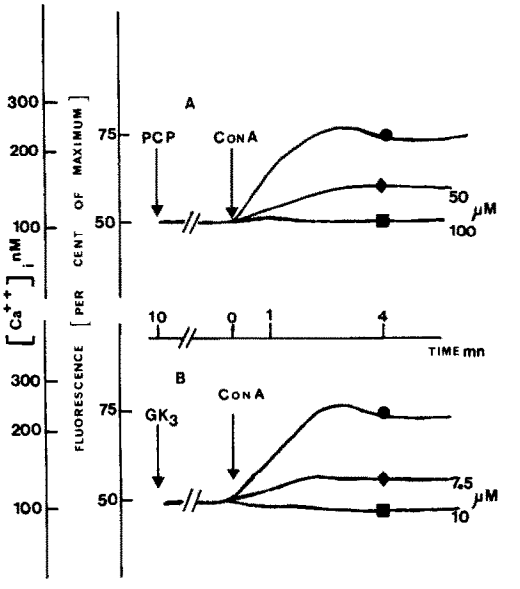


Fig. 3. Inhibitory effect of PCP and GK_3 on Con A induced $[Ca^{2+}]_i$ tise in mouse thymocytes. Thymocytes were loaded with Quin-2-AM and their fluorescence monitored (λ_{ex} 339 nm, λ_{em} 492 nm). When indicated (arrow), 10 μg/ml Con A was added to the cell suspension (time 0). $[Ca^{2+}]_i$ was calculated [19] and plotted as a function of time (●). Curve A: 50 μM (◆) or 100 μM (■) PCP was added to the cell suspension 10 min before Con A. Curve B: 7.5 μM (◆) or 10 μM (■) GK_3 was added to the cell suspension 10 min before Con A.

3) and determined the effects of different drugs, Con A being added to the cell suspension 10 min after the drugs. Figure 3 (A and B) shows that PCP and GK_3, acting in a dose-dependent manner, prevented the calcium rise induced by the lectin. Similar marked inhibition was obtained with other PCP derivatives. All these compounds equally block Con A-induced $[Ca^{2+}]_i$ rise in splenocytes and HMC. Following removal of the drug by washing, addition of Con A stimulated a normal increase of $[Ca^{2+}]_i$, confirming the reversibility of the effects of PCP or analogs (data not shown). For some drugs, Table 4 reports the concentrations leading to 50% inhibition of the Con A-induced $[Ca^{2+}]_i$ rise. It shows that the ability of these compounds to inhibit lymphocyte proliferation paralleled their ability to block the $[Ca^{2+}]_i$ rise required for IL2 secretion, even if this second effect is observed at slightly higher concentrations.

6. *Effect of PCP or analogs on lymphocyte membrane potential*

$[Ca^{2+}]_i$ changes induced by lectins are dependent on membrane potential: a depolarization of the membrane can reduce $[Ca^{2+}]_i$ and the proliferative response to mitogenic lectins [27]. Using a fluorescent potential-sensitive dye, DiI-C_1 [5], we measured thymocyte membrane potential as previously described [20], in the presence or in the absence of PCP or GK_3. Figure 4 shows that addition of 7.5 μM GK_3 to thymocytes immediately depolarized these cells: the membrane potential changed from its resting level, approximately −64.5 mV, to −43 mV. Under the same conditions the small Con

Table 4. Inhibitory effect of PCP and some of its congeners on Con A induced proliferation of murine thymocytes and on Con A induced $[Ca^{2+}]_i$ rise in the same cells

	GK_0	GK_1	GK_3	GK_4	GK_{13}	GK_{67}	GK_{144}
IP_{50}	30 ± 18	48 ± 10	2.5 ± 1.1	15 ± 5	8 ± 3	4.3 ± 1.5	0.6 ± 0.2
ICa_{50}	50 ± 20	75 ± 15	6.0 ± 1.5	18 ± 3	13 ± 2	8.0 ± 0.5	1.2 ± 0.5

Comparison of drug concentrations (μM) promoting half inhibition of Con A induced proliferation (IP_{50}) or Con A induced $[Ca^{2+}]_i$ rise (ICa_{50}). Mean of three different experiments.

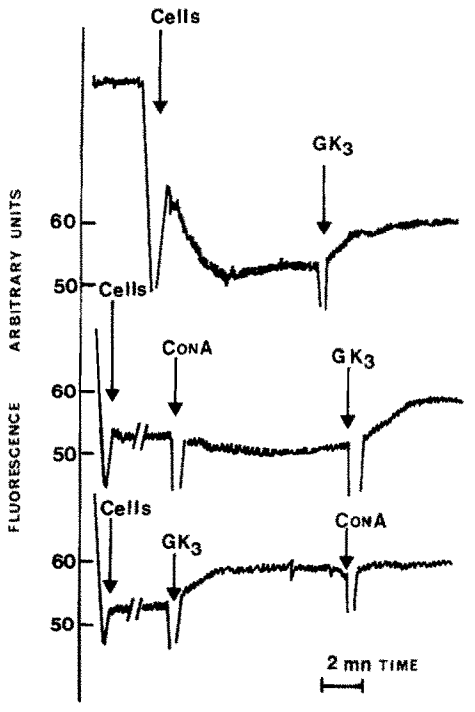


Fig. 4. Effect of GK_3 on thymocyte membrane potential. Thymocytes (5×10^6 cells/ml) were preequilibrated in the buffer indicated in Materials and Methods, containing 0.5 μM DiI-C_1-(5), a fluorescent membrane potential indicator. When indicated (arrow) 7 μM GK_3 or 10 μg/ml Con A was added to the cell suspension (time 0). The fluorescence of the suspension was monitored (λ_{ex} 620 nm, λ_{em} 660 nm). In separate experiments the membrane potential in absence or presence of additives were calculated by the null point method [20].

A induced hyperpolarization (−7 mV) could not be evidenced and the addition of GK_3 after Con A completely abolished the lectin effect and depolarized the cell membrane (Fig. 4).

At this low concentration PCP did not affect lymphocyte membrane potential, but 100 μM PCP displayed the same depolarizing action (data not shown). We also obtained that, like GK_3 or PCP, all the other PCP congeners exert a depolarizing action on thymocytes, splenocytes or HMC membranes at concentrations preventing the lectin-induced $[Ca^{2+}]_i$ rise (data not shown).

DISCUSSION

Our study shows that the proliferative response of murine or human T lymphocytes to mitogenic lectins is decreased when these cells are treated *in vitro* by high doses of PCP or TCP (>20 μM) at the onset of the activation. Unlike these compounds the general anesthetic ketamine does not exert the same suppressive effect, which suggests that it most probably does not affect T lymphocyte functions *in vivo*.

The other PCP (or TCP) derivatives that we tested also diminish T lymphocyte blastogenesis in a dose-dependent manner and are more potent inhibitors of this lymphocyte response than the parent compounds: half inhibition was obtained between 0.2 and 10 μM, as compared with 40–50 μM for PCP or TCP. The inhibition does not result from a toxicity of the drugs and the fact that extensive washing restores the lymphocyte capacity to respond to a mitogenic stimulus suggests a direct interaction of the drugs with the external face of the cell membrane, maybe with specific receptor site(s).

The fact that PCP and its congeners block an early event of T lymphocyte activation also explains why the murine IL-2 dependent CTLL line which represents primed lymphoid cells proliferates in presence of drug concentations which completely suppress thymocyte or splenocyte proliferation.

Cytokine synthesis occurring soon after the mitogenic stimulus is an obligatory step in lymphocyte proliferation [22]. Our data showing that PCP does not alter IL-1 production from LPS-activated murine macrophages suggest that these cells are not the target of the inhibitory action of the drug, a result conflicting with that of Khansari *et al.* [4] who observed that PCP treatment reduced IL-1 production of human monocytes by 50%. We cannot explain the discrepancy between these authors' data and ours since we have repeated our results with all the PCP derivatives tested (Table 1): all failed to inhibit IL-1 production even at concentrations 2–3-fold higher than those preventing thymocyte or splenocyte responses. These data favour the absence of interaction between macrophages and PCP.

Conversely, at concentrations preventing mitogenesis, all the drugs strongly inhibit IL-2 accumulation in the supernatants of Con A stimulated splenocytes. This effect, in the absence of inhibition of IL-1 synthesis, is sufficient by itself to explain the suppression of lymphocyte proliferation and shows that a T helper cell function is affected by the drug action. Since IL-2 production by JURKAT cells is also similarly suppressed, one can imagine that a general biochemical mechanism involved in IL-2 production is affected by the drugs. Comparison of the inhibitory effects of GK_3 on murine thymocytes or splenocytes stimulated by either Con A, or PHA + PMA, or PMA + A23187, suggests that the drug interacts with the calcium signal delivered by the lectin binding to its receptor(s); effectively, lymphocyte activation by the calcium ionophore [24, 26] which by-passes this signal is little affected, while the response observed in the presence of the protein kinase C activator PMA and the lectin is still inhibited.

When using the fluorescent indicator Quin-2, we found, like others [18], that Con A rapidly elevates $[Ca^{2+}]_i$ in thymocytes. In contrast, when Quin-2-loaded thymocytes are pretreated with PCP or analogues before lectin addition, we noticed a marked inhibition of this enhancement. Half inhibition of the $[Ca^{2+}]_i$ rise is observed at drug concentrations slightly higher than those inhibiting IL-2 synthesis and lymphocyte proliferation, a difference which probably arises from the different conditions for the two types of experiment. However, we observed a similar potency order for the drugs in calcium rise inhibition and in cell proliferation inhibition. It is well defined that the $[Ca^{2+}]_i$ rise in mitogen-treated lymphocytes is an intracellular messenger for IL-2

production [28] but not for the IL-2 induced proliferation [29]; thus our data tend to establish that PCP and its analogs which do not affect primed cells prevent IL-2 synthesis by inhibiting the obligatory mitogen-induced $[Ca^{2+}]_i$ rise; the efficiency of each drug for interacting with this biochemical signal is different.

Our data show that the drugs affect not only T helper cells involved in IL-2 production but also immature thymocytes: the Con A induced $[Ca^{2+}]_i$ rise of unseparated thymocytes is due to the sum of the responses of the immature cortical population and of the mature medullary population which represent 75–85% and 10–20% of total thymocytes, respectively, and Con A induces similar changes of $[Ca^{2+}]_i$ in both subsets [30]. Despite these results it is unlikely that PCP derivatives affect similarly all T lymphocyte populations: indeed, when stimulated by PMA + A23187, splenocytes were more sensitive than thymocytes to GK_3 effects (Fig. 2), which suggests that, in addition to the effect on $[Ca^{2+}]_i$ rise observed on both populations, the drug might also interact with other early events of the activation, in a manner different in each population.

In lymphocytes the lectin-triggered $[Ca^{2+}]_i$ changes are dependent on membrane potential: depolarization of the membrane reduced $[Ca^{2+}]_i$ and the proliferative response to lectins [28]. Our preliminary results showing that PCP and congeners depolarize the membrane of resting thymocytes and prevent the hyperpolarization of the stimulated cells could explain the ability of these drugs to prevent $[Ca^{2+}]_i$ rise; this might imply an interaction between the drugs and cation channels. PCP has been reported to block K^+ channels in CNS [31] and could exert a similar effect on lymphoid cells. Two K^+ channels with different characteristics have been described in these cells [8, 32, 33]. Other cation channels, such as the Na^+ channel [8], could also be implicated. Moreover, it is well known that the $[Ca^{2+}]_i$ increase in lymphocytes after antigen triggering is due to a great extent to extracellular calcium influx [23, 34] which does not occur through the voltage-sensitive K^+ channels [35] as previously believed [36]. This increase is not observed in the presence of calcium channel blockers, such as nifedipine [37]; a nifedipine-like activity of the PCP drugs could explain the suppression of the $[Ca^{2+}]_i$ rise. Measurements of cation transport and associated electric currents in resting or stimulated lymphocytes, in the presence or absence of drugs, might help to explain how PCP and derivatives affect membrane potential and Con A induced $[Ca^{2+}]_i$ increase.

Except ketamine, all PCP derivatives inhibit IL-2 synthesis and $[Ca^{2+}]_i$ rise; it appears difficult to establish structure–activity relationships with the small number of compounds tested. However, some preliminary inferences can be drawn from our data. PCP (GK_1) and TCP (GK_0) are the least potent inhibitors; they are also the molecules with the most mobile conformation. The molecules which have been slightly modified by cyclohexyl substitutions, GK_4, GK_6, GK_{18}, are 3–4-fold more efficient than the original compound GK_1, whereas those which have received an equatorial tertiobutyl-4 group that blocks the molecule in a quasi-rigid conformation with an axial phenyl group exert the most inhibitory action: GK_3 and GK_{117} are 10–20-fold more inhibitory than GK_1, GK_{67} 6–9-fold more than GK_0. These results seem to establish the necessity of an increased rigidity of the molecular structure for maximal inhibitory activity (possibly together with an increased lipophilicity). When we compare GK_{13} to GK_1 and GK_{138}, GK_{144}, GK_{145}, GK_{154}, GK_{155} to GK_3, we find that this activity is also affected by modifications of the aromatic group. In fact it appears that the addition of electron donating substitutions of the phenyl ring (GK_{138}, GK_{144}, GK_{145}) which enhances the basicity of the molecule, increases more efficiently the inhibitory action of the drugs than the electron withdrawing substitutions (GK_{154}, GK_{155}) which decrease the basicity. Other structural modifications could give rise to more active compounds but the necessity of a rigid conformation with an axial phenyl group and increased basicity appears likely: GK_{144} and GK_{145} exert the same inhibitory effect as PCP but at concentrations 150–200-fold lower.

The existence of PCP receptors on T lymphocyte is questionable; it has been proposed that a great heterogeneity of these receptors exists in these cells [4]. Previous studies [38] have established that PCP derivatives with a rigid conformational structure, like GK_3, possess a low affinity (100-fold lower than GK_1) for [^{3}H]-PCP sites in the CNS and display few behavioral effects. Thus the molecules which are the least potent on lymphocytes are the most active on the CNS and vice versa. This suggests that PCP does not exert its suppressive effect on lymphocytes by interacting with PCP-binding entities similar to those in nervous cells, such as the opiate sigma membrane receptor [39] or the 43 kD and 95 kD synaptosome proteins, or the α and β subunits of the acetylcholine receptor [4, 12, 40]. It seems rather likely that it interacts with cell membrane entities having a high affinity for the quasi-rigid PCP analogs which could be cation channels as suggested by membrane potential determinations.

The structural modifications of the PCP molecule have completely changed its initial properties; they have given rise to a class of compounds which could be useful tools for studying the cation channels involved in lymphocyte proliferation and which have immunosuppressive properties. On the other hand, the study of the effects of PCP congeners on some particular events involved in lymphocyte activation might constitute an interesting comparative model for understanding the action of PCP at the CNS level.

REFERENCES

1. R. C. Petersen and R. C. Stillman (Eds.), *Phencylcidine (PCP) Abuse: an Appraisal*, Vol. 21. NIDA Research Monographs, US Government Printing Office, Washington, DC (1978).
2. E. F. Domino (Ed.). *PCP (Phencyclidine): Historical and Current Perspectives.*, NPP Books, Ann Arbor, MI (1981).
3. J. M. Kamenka, E. F. Domino and P. Geneste (Eds.), *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications*. NPP Books, Ann Arbor, MI (1983).

4. N. Khansari, H. D. Whitten and H. H. Fudenberg, *Science* **225**, 76 (1984).
5. E. F. Domino and E. D. Luby, in *Psychopathology and Psychopharmacology*, (Eds. J. O. Cole, A. M. Freedman and A. J. Friedhoff), pp. 37–50. John Hopkins University Press, Baltimore, MD (1973).
6. H. H. Fudenberg, H. D. Whitten, E. Merler and D. Farmati, *Med. Hypoth.* **12**, 85 (1983).
7. J. G. Kaplan, *Ann. Rev. Physiol.* **40**, 19 (1978).
8. E. K. Gallin, *J. Leukocyte Biol.* **39**, 241 (1986).
9. J. Dornand, J. Favero, J. C. Bonnafous and J. C. Mani, *Immunobiol.* **171**, 436 (1986).
10. Y. Tourneur, G. Romey and M. Lazdunski, *Brain Res.* **245**, 154 (1982).
11. E. S. Albuquerque, M. C. Tsai, R. S. Aronstam, A. T. Eldefrani and M. E. Eldefrani, *Molec. Pharmac.* **18**, 167 (1980).
12. Y. Kloog, A. Kalir, O. Bucaman and M. Sokolovsky, *FEBS Lett.* **109**, 125 (1980).
13. P. Geneste, J. M. Kamenka, S. N. Ung, P. Herrmann, R. Goudal and G. Trouiller, *Eur. J. Med. Chem.* **14**, 301 (1979).
14. M. Mousseron, J. M. Kamenka and M. R. Darvich, *Bull. Soc. Chim. Fr.* 1435 (1970).
15. J. Dornand, J. Favero, J. C. Bonnafous and J. C. Mani, *J. Cell. Immunol.* **83**, 351 (1984).
16. J. Dornand, J. Favero, J. C. Bonnafous and J. C. Mani, *Biochem. Med.* **28**, 144 (1982).
17. O. Abelhsira-Amar, C. Damais, M. Parant and L. Chedid, *J. Immunol.* **134**, 365 (1985).
18. R. T. Hesketh, S. Bavetta, G. A. Smith and J. C. Metcalfe, *Biochem. J.* **214**, 575 (1983).
19. T. J. Rink, R. Y. Tsien and T. Pozzan, *J. Cell Biol.* **95**, 189 (1982).
20. T. J. Rink, C. Montecucco, T. R. Hesketh and R. Y. Tsien, *Biochim. biophys. Acta* **595**, 15 (1980).
21. J. M. Kamenka, M. Michaud and P. Geneste, *Eur. J. Med. Chem.* **20**, 419 (1985).
22. J. R. Robb, A. Munck and K. A. Smith, *J. exp. Med.* **154**, 1455 (1981).
23. J. B. Imboden and J. D. Stobo, *J. exp. Med.* **161**, 446 (1985).
24. A. Truneh, F. Albert, P. Golstein and A. M. Schmitt-Verhulst, *Nature, Lond.* **313**, 318 (1985).
25. D. Delia, M. Greaves, S. Villa and F. D. De Brand, *Eur. J. Immunol.* **14**, 720 (1984).
26. O. Josimovits, H. Osawa and T. Diamantstein, *Immunobiol.* **170**, 164 (1985).
27. E. W. Gelfand, R. K. Cheung and S. Grinstein, *J. Cell. Physiol.* **121**, 533 (1984).
28. G. B. Mills, R. K. Cheung , S. Grinstein and E. W. Gelfand, *J. Immunol.* **134**, 1640 (1985).
29. G. B. Mills, R. K. Cheung, S. Grinstein and E. W. Gelfand, *J. Immunol.* **134**, 2431 (1985).
30. C. El Moatassim, J. Dornand and J. C. Mani, *Biochim. biophys. Acta* **927**, 437 (1987).
31. M. P. Blanstein and R. K. Ickowicz, *Proc. natn. Acad. Sci. U.S.A.* **80**, 3835 (1983).
32. M. D. Cahalan, K. G. Chandry, T. E. De Coursey and S. A. Gupta, *J. Physiol., Lond.* **358**, 197 (1985).
33. T. E. De Coursey, K. G. Chandry, S. A. Gupta and M. D. Cahalan, *Nature, Lond.* **307**, 465 (1984).
34. A. Alcover, M. J. Weiss, J. F. Daley and E. L. Reinhertz, *Proc. natn. Acad. Sci. U.S.A.* **83**, 2614 (1986).
35. E. W. Gelfand, R. K. Cheung and S. Grinstein, *J. biol. Chem.* **261**, 11520 (1986).
36. K. G. Chandry, T. E. De Coursey, M. D. Cahalan, C. McLaughlin and S. A. Gupta, *J. exp. Med.* **160**, 369 (1984).
37. R. K. Cheung, S. Grinstein, H. M. Dosch and E. W. Gelfand, *Eur. J. Immunol.* **16**, 907 (1986).
38. J. P. Vincent, R. Kartolovski, P. Geneste, J. M. Kamenka and M. Lazdunski, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4678 (1979).
39. J. P. Vincent, D. Cavey, J. M. Kamenka, P. Geneste and M. Lazdunski, *Brain Res.* **152**, 176 (1978).
40. A. L. Goldstein, B. Haber and G. H. Cohen, in *Neurochemical and Immunologic Components in Schizophrenia* (Eds. D. Bergama and A. L. Goldstein), pp. 1–20. Liss, New York (1978).