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SHORT COMMUNICATIONS

Effects of perphenazine on the metabolism of inositol phospholipids in SK-N-BE(2) human neuroblastoma cells

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Abstract—Administration of *myo*-[^{3}H]inositol to SK-N-BE(2) human neuroblastoma cells for 24 hr resulted in equilibrium labelling of phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2), as well as in retention of a large intracellular pool of free *myo*-[^{3}H]inositol. Equilibrium labelling was no longer observed when cells were treated for 2 hr with 20 μM perphenazine (PPZ) in label-free medium; under these conditions, *myo*-[^{3}H]inositol from the retained intracellular pool was incorporated into PI and PIP but not into PIP_2. Analysis of water-soluble *myo*-[^{3}H]inositol derivatives and inositol 1,4,5-trisphosphate mass determination indicated that PPZ did not stimulate phosphoinositide hydrolysis by phospholipase C. These results indicate that PPZ raises PI and PIP levels, whereas it is ineffective in expanding the PIP_2 pool. The latter effect is not due to a concomitant synthesis and hydrolysis of this lipid.

Key words: perphenazine; inositol phospholipids; neuroblastoma

It is well established that phenothiazines potently stimulate PI* synthesis, mainly through impairment of phosphatidate phosphohydrolase activity [1]; however, the effects of these neuroleptic drugs on polyphosphoinositide metabolism are more controversial [2–5]. The evidence that phenothiazines may affect the activity of PI kinase [6] and PIP kinase [7] suggests that PIP and PIP_2 synthesis is not clearly enhanced as a consequence of increased substrate availability for the above mentioned enzymes. Furthermore, it has been suggested that phenothiazines may also stimulate phosphoinositide hydrolysis by phospholipase C [8]. Until now, studies dealing with the effects of phenothiazines on phosphoinositide metabolism have been largely carried out on non-neural cell systems. Herein we address the issue of PPZ effects on phosphoinositide metabolism employing the human neuroblastoma cell line SK-N-BE(2) as a model system.

Materials and Methods

Plastics for cell culture, MEM, RPMI 1640 and foetal calf serum were from Flow Lab. (Herts, U.K.). *Myo*-[2-^{3}H]inositol (17.5 Ci/mmol) was from Amersham Corp. (Amersham, U.K.). Perphenazine hydrochloride and Dowex-1 chloride were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). The SK-N-BE(2) human neuroblastoma cell line, obtained by courtesy of Dr G. Melino (University of Rome "Tor Vergata", Italy), was cultured as described previously [9] and used at the 29–35th passage. Cells grown in 25 cm^2 flasks were labelled with 12.5 μCi of *myo*-[^{3}H]inositol in 2.5 mL of MEM plus 10% fetal calf serum. After 24 hr labelling cells were washed with medium (MEM) and processed immediately or after reincubation for 2 hr at 37° in medium, under the conditions indicated in the text. After addition of cold 10% trichloroacetic acid, acid-soluble [^{3}H]inositol metabolites were extracted and separated by anion-exchange chromatography on Dowex-1 as described [10]. [^{3}H]Phosphoinositides were extracted from the acid-insoluble pellet and, after deacylation, separated as glycerophosphoderivatives by ion-exchange chromatography as previously reported [10]. 1,4,5-IP_3 mass was measured by a commercially available specific radioreceptor assay (Amersham), as described [10]. Radioactivity was measured with a Packard Tricarb 1500 scintillation counter, using Optifluor (Packard) as the scintillation fluid. Protein determination was carried out according to [11].

Results and Discussion

Long-term cell exposure to *myo*-[^{3}H]inositol, under the conditions described, resulted in extensive labelling of inositol phospholipids (Table 1) as well as in retention of a large intracellular pool of free *myo*-[^{3}H]inositol (1.9×10^6 dpm/mg protein). In spite of the presence of such a large pool of radioactive precursor, cell reincubation in label-free medium for 2 hr did not change the radioactivity associated with any of the individual classes of inositol phospholipids, thus indicating achievement of equilibrium labelling (Table 1). Table 1 shows that equilibrium conditions did not last longer when cells were reincubated in the presence of 20 μM PPZ. Under these conditions, PPZ stimulated the incorporation of free intracellular *myo*-[^{3}H]inositol into PI and PIP but not into PIP_2. The effect of PPZ, observed when isotopic labelling of inositol phospholipids had already attained equilibrium, support the view that changes in lipid labelling may reflect actual changes in phosphoinositide intracellular levels. In an attempt to explain the evidence that expansion of the PIP pool is not a sufficient condition to enhance PIP_2 levels, we investigated whether PPZ elicits PIP_2 hydrolysis by phospholipase C. Thus, cells prelabelled with *myo*-[^{3}H]-inositol for 24 hr were reincubated for 2 hr in medium containing 10 mM Li^+ in the presence or absence of 20 μM PPZ, and accumulation of water-soluble hydrolysis products monitored. Table 2 shows that PPZ enhanced the accumulation of GPI, a metabolite arising from full PI deacylation, but not of the individual classes of inositol phosphates, generated by phospholipase C hydrolysis. In addition, a set of experiments which are not shown indicated

* Abbreviations: PPZ, perphenazine; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; 1,4,5-IP_3, inositol 1,4,5-trisphosphate; GPI, glycerophosphoinositol.

Table 1. PPZ-evoked changes in phosphoinositide labelling, due to incorporation of intracellular free *myo*-[^{3}H]inositol

	Radioactivity (dpm × 10^{-3}/mg protein)		
	PI	PIP	PIP_2
Not reincubated	263.5 ± 28.2	12.9 ± 1.4	9.0 ± 1.0
Reincubated:			
without PPZ	266.2 ± 27.9	13.0 ± 1.3	9.0 ± 1.1
with 20 μM PPZ	355.6 ± 32.6*	17.2 ± 1.5*	7.5 ± 0.9

Cells were labelled for 24 hr with *myo*-[^{3}H]inositol and processed immediately after washing (not reincubated) or reincubated for 2 hr in label-free medium, in the presence or absence of 20 μM PPZ. At the beginning of reincubation the radioactivity associated with the intracellular free *myo*-inositol pool was 1.9×10^6 dpm/mg protein. Inositol phospholipids were extracted and separated as described in the text.

Each value is the mean ± SD of four independent determinations. * $P < 0.01$, versus samples reincubated without PPZ, as from Student's *t*-test.

Table 2. PPZ effect on the accumulation of water-soluble *myo*-[^{3}H]inositol metabolites produced upon phosphoinositide hydrolysis

	Radioactivity (dpm × 10^{-3}/mg protein)	
	Reincubated without PPZ	Reincubated with 20 μM PPZ
GPI	7.7 ± 0.9	12.4 ± 1.0*
IP	10.1 ± 1.6	11.5 ± 1.8
IP_2	1.5 ± 0.4	2.0 ± 0.5
IP_3	0.5 ± 0.2	0.5 ± 0.3
IP_4	0.4 ± 0.2	0.5 ± 0.2

Cells were labelled for 24 hr with *myo*-[^{3}H]inositol, washed and reincubated for 2 hr in label-free medium containing 10 mM Li^+, in the presence or absence of 20 μM PPZ. Water-soluble products of phosphoinositide hydrolysis were extracted and separated as described in the text. Inositol mono- (IP), bis- (IP_2), tris-(IP_3) and tetrakis- (IP_4) phosphates were not resolved into individual isomers.

Each value is the mean ± SD of four independent determinations. * $P < 0.01$, versus samples reincubated without PPZ, as from Student's *t*-test.

that cell incubation in the presence of 20 μM PPZ did not alter 1,4,5-IP_3 mass from the basal value of 7.6 (± 3.5) pmol/mg cell protein, as monitored at 15 sec, 30 sec, 5 min, 30 min, 1 hr and 2 hr after drug addition. While increased GPI labelling may reflect activation of homeostatic mechanisms through which cells respond to increased PI synthesis, it is clear that the failure of PPZ to stimulate *myo*-[^{3}H]inositol incorporation into PIP_2 is not due to a concomitant synthesis and hydrolysis of this lipid. These results show that in SK-N-BE(2) cells, PPZ not only enhances PI synthesis but also directs polyphosphoinositide metabolism towards expansion of the PIP pool. Studies on bovine adrenal medulla preparations have demonstrated that phenothiazines stimulate PI kinase but inhibit PIP kinase [6, 7]. Such effects of PPZ on the above mentioned enzymes could drive PIP accumulation in the cell system investigated here. Elevation of the PIP steady-state level has also been reported to occur in human platelets after treatment with chlorpromazine at non-permeabilizing concentration [5]; incidentally, in the present study, PPZ was employed at a concentration below its critical micellar concentration [12]. It remains to be investigated whether the possibility that phenothiazines may selectively expand the PIP pool over the PIP_2 is of pharmacological relevance. At the moment, it can be observed that since PIP and PIP_2 are equally good substrates for receptor-activated phospholipases C [13], cell stimulation in the presence of phenothiazines is expected to result in a predominant hydrolysis of PIP over PIP_2, thus leading to unbalanced production of diacylglycerol and 1,4,5-IP_3.

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