Effect of deoxycholate on guanine-nucleotide-dependent carbachol stimulation of phosphoinositidase C in mouse brain cortical membranes

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Demonstration of guanine-nucleotide-dependent neurotransmitter stimulation of phosphoinositide breakdown in brain membranes has generally required the presence of the detergent, deoxycholate (DOC), in the assay medium. In the present study, by using mouse brain cortical membranes labelled with [^3H]inositol in the presence of CMP through the reverse PtdIns synthase reaction, we have been able to show guanosine 5'-[γ -thio]triphosphate (GTP[S])-dependent carbachol (CCh) stimulation of the formation of [^3H]inositol phosphates in the absence of DOC and have studied how the detergent affects the response. The results of our study indicate that DOC affects the muscarinic receptor–G-protein–phosphoinositidase C (PIC) transduction system in several ways. First, it enhances agonist-induced PIC

activity towards [^3H]PtdInsP and [^3H]PtdInsP₂ and, secondly, it decreases the potency for GTP[S] stimulation of PIC, thus enhancing the agonist-induced leftward shift of the dose–response curve for GTP[S]. Additionally, DOC appears to increase the activity of the enzymes of the phosphoinositide cycle, PtdIns 4-kinase, Ins(1,4,5)P₃ 5-phosphatase and Ins(1,4)P₂ 1-phosphatase, thus altering the proportion of phosphoinositide substrates and inositol phosphate products. These observations advise caution in drawing conclusions about PIC substrate specificity and the potency of both guanine nucleotides and agonists from experiments performed in membranes in the presence of DOC or related bile salts.

INTRODUCTION

The interaction of a large variety of hormones and neurotransmitters with specific membrane receptors promotes the hydrolysis of inositol phospholipids through activation of phosphoinositidase C (PIC) with the generation of two second messengers, Ins(1,4,5)P₃ and *sn*-1,2-diacylglycerol, which mobilize calcium from intracellular stores and activate protein kinase(s) C respectively [1,2]. Several PIC isoenzymes have been identified and at least three classes, termed β , γ and δ , have been clearly defined on the basis of differences in the relative positions of conserved domains within the primary structure and differential reactivity to monoclonal antibodies [3]. Only the β -isoform appears to be activated by G-proteins that are members of a family of pertussis-toxin-insensitive G-proteins, the G_q class [4,5]. In brain tissue, hydrolysis-resistant guanine nucleotide analogues and AlF₄[−] readily stimulate the PIC activity in membrane preparations [6,7]. However, in contrast with other tissues, the demonstration of GTP-dependent activation of phosphoinositide hydrolysis by agonists in brain membranes has proved more difficult and has generally required the presence of the detergent, deoxycholate (DOC) in the assay medium, whether [^3H]inositol-prelabelled membranes [8] or exogenous [^3H]phosphoinositides [9] were used as substrates. The reason for this detergent requirement is not well understood, and possible detergent effects upon different enzymes of phosphoinositide metabolism or the coupling of the transduction system components have not been investigated. In the present study, by using membranes labelled with [^3H]inositol in the presence of CMP through the reverse PtdIns synthase reaction [10], we have been able to compare the stimulation of phosphoinositide hydrolysis in the absence and presence of DOC in membranes of mouse cerebral cortex. A preliminary account of this work has been published in abstract form [11].

MATERIALS AND METHODS

Materials

D-*myo*-[^3H]inositol (18.3 Ci/mmol) and D-*myo*-[^3H]Ins(1,4,5)P₃ (55 Ci/mmol) were obtained from Amersham International; guanosine 5'-[γ -thio]triphosphate (GTP[S]) was from Boehringer Mannheim; Dowex AG-1-X8 (100–200 mesh; formate form) was from Bio-Rad; CMP, ATP, carbachol (CCh) and EGTA were from Sigma; DOC was from Merck.

Preparation and labelling of membranes

Hybrid B6CBAF1 mice were used. Brain cortical membranes were prepared as described previously [8]. In brief, brain cortices were homogenized in 10 vol. of ice-cold 20 mM Tris/HCl/1 mM EGTA, pH 7.0, by using a motor-driven Dounce homogenizer with a Teflon pestle (20 strokes at 800 rev./min). Homogenates were centrifuged for 15 min at 39 000 *g*; pellets were washed three times and stored at −80 °C until required for use. Membranes were labelled with [^3H]inositol in the presence of CMP by a modification of the method described by Claro et al. [12]. Frozen membranes (10–15 mg of protein/ml) were resuspended in 20 mM Tris/HCl, pH 7.0, containing 1 mM EGTA, 6 mM MgCl₂, 2 mM ATP, 1 mM CMP and [^3H]inositol (8–10 $\mu\text{Ci/ml}$) and incubated at 37 °C. After 120 min, membranes were centrifuged at 14 000 *g* for 25 min, and the pellets obtained were resuspended by sonication in 25 mM Tris/maleate, pH 6.8.

Phospholipase C assay

Aliquots of resuspended membranes (150 μg of protein) were incubated for the indicated times at 37 °C in 100 μl of the same buffer containing (in mM): MgCl₂ 6, ATP 2, EGTA 3, CaCl₂ 0.27 (100 nM free calcium) and, when present, GTP[S] and agonists. In some experiments this procedure was scaled up 2.5-

fold. Incubations were stopped by addition of 1.2 ml of chloroform/methanol (1:2, v/v). A 0.5 ml portion of both 0.25 M HCl and chloroform were added to the tubes and the phases were separated. A 1 ml portion of the upper phase was neutralized with 1.5 M NH_4OH , diluted with 4 ml of distilled water and passed through columns of Dowex AG-1-X8 (formate form) of bed volume 0.5 ml [8]. Experiments were performed in triplicate. For determination of isomeric [^3H]inositol phosphates by HPLC, incubations were stopped by addition of 100 μl of 1 M trichloroacetic acid. Precipitated protein was removed by centrifugation, and 150 μl of supernatant freed of acid by washing with 5 $\times$ 1 ml of water-saturated diethyl ether and neutralized with 1.5 M NH_4OH . Triplicate tissue extracts were pooled for analysis of reaction products with a Partisil 10 SAX column (Technicol) using an ammonium phosphate (adjusted to pH 3.7 with phosphoric acid) gradient [9]. Separation and analysis of [^3H]phosphoinositides was performed by alkaline deacylation of pooled triplicate samples, followed by separation of the water-soluble products by Dowex anion-exchange chromatography as described by Godfrey [13].

Analysis of data

Statistical analysis of results was achieved by using the paired or unpaired Student's *t* test ($P < 0.05$). Concentration-effect curves were fitted to the Hill equation using a non-linear-regression program.

RESULTS

Initial studies using both rat and mouse cortex showed that direct labelling of membranes with [^3H]inositol in the presence, but not in the absence, of 1 mM CMP resulted in a higher incorporation of label into phosphoinositides (up to 10% of added [^3H]inositol) than in membranes prepared from [^3H]inositol-prelabelled slices (2–3%). When GTP[S]-dependent CCh stimulation of [^3H]phosphoinositide hydrolysis was examined in membranes labelled with [^3H]inositol in the presence of CMP, a small but significant potentiation of the nucleotide effect by the agonist was observed in the absence of DOC. As shown in Table 1, a 15 min incubation with 1 μM GTP[S] induced both

[^3H]InsP and [^3H]InsP₂ accumulation in the absence (146 and 754% over basal respectively) and presence (239 and 520%) of 1 mM DOC. CCh (1 mM) alone had no effect in either condition (results not shown). However, it significantly potentiated the nucleotide effect, thereby stimulating [^3H]InsP and [^3H]InsP₂ accumulation with respect to basal levels by 203 and 923% respectively without DOC and 665 and 1212% respectively with DOC. In the presence of the detergent there was an enhancement of agonist-stimulated formation of both [^3H]inositol phosphates, but the potentiating effect was 2-fold greater for [^3H]InsP (the [^3H]InsP/[^3H]InsP₂ ratio was 0.64 ± 0.03 without DOC; 1.17 ± 0.10 with DOC; mean $\pm$ S.E.M., $n = 5$). Since no significant accumulation of [^3H]InsP₃ was observed under these conditions, the normal assay was scaled up 2.5-fold to study the effect of DOC on the kinetics of [^3H]InsP, [^3H]InsP₂ and [^3H]InsP₃ formation (Figure 1). GTP[S]-dependent CCh stimulation of [^3H]InsP formation was not evident before 2 min and increased linearly thereafter until at least 15 min, whether DOC was present or not. In contrast, [^3H]InsP₂ and [^3H]InsP₃ were readily measurable at 30 s, and their levels increased linearly during shorter times (5 min for [^3H]InsP₂ and 2–5 min for [^3H]InsP₃). In the absence of DOC, the initial rate for agonist-stimulated production of [^3H]InsP₂ was higher than that of [^3H]InsP (1500 and 650 d.p.m./min respectively). In the presence of the detergent, there was a 2.6-fold increase in the initial rate of [^3H]InsP₂ formation (3950 d.p.m./min) and a higher increase (3.4-fold) in the initial rate of formation of [^3H]InsP (2200 d.p.m./min). Rates of [^3H]InsP₃ accumulation were similar under both conditions up to 2 min (410 and 570 d.p.m./min in the absence and presence of DOC respectively), but after that time the rate in the presence of DOC was higher and steady-state levels of [^3H]InsP₃ at 15 min were two times higher than in the absence of DOC. Taken together these results suggested that, apart from increasing PIC activity, DOC could also be affecting other aspects of phosphoinositide metabolism.

In order to investigate this possibility, we analysed the levels of [^3H]phosphoinositides. As shown in Table 2, basal levels of [^3H]PtdInsP increased almost 2-fold in the presence of DOC, whereas those of [^3H]PtdInsP₂ and [^3H]PtdIns were not significantly modified. Stimulation with GTP[S] plus CCh induced a significant decrease in both [^3H]PtdInsP and [^3H]PtdInsP₂ with respect to basal levels, whether DOC was present or not. This effect was greater in the presence of the detergent, especially in the case of [^3H]PtdInsP [12.0 ± 2.5 and $30.4 \pm 2.8\%$ decrease in [^3H]PtdInsP; 20.3 ± 2.6 and $28.4 \pm 2.0\%$ decrease in [^3H]PtdInsP₂, without and with DOC respectively ($n = 7$, paired Student's *t* test)]. Additionally, there was a significant decrease in [^3H]PtdIns only in the presence of DOC ($10.5 \pm 2.0\%$; $n = 7$) (Table 2). This could be explained by an increase in the [^3H]PtdInsP turnover, but it could also be indicative of a change in the substrate specificity of PIC. Since hydrolysis of [^3H]PtdIns will lead to the formation of [^3H]Ins1P, whereas hydrolysis of [^3H]PtdInsP₂ and/or [^3H]PtdInsP will give rise to [^3H]Ins(1,4)P₂ and [^3H]Ins4P [16], we then analysed the [^3H]inositol phosphate isomers accumulated, by the use of HPLC. As shown in Table 3, GTP[S] alone and in the presence of CCh induced a small, but significant, formation of [^3H]Ins1P only when DOC was present (61 and 154% increase over basal respectively), suggesting that the detergent allows PIC to hydrolyse some [^3H]PtdIns. However, under this condition, there was also a large increase in the accumulation of the [^3H]Ins4P isomer, which is consistent with the increased [^3H]polyphosphoinositide turnover observed in Table 2. Additionally, the ratio of [^3H]Ins4P to [^3H]Ins(1,4)P₂ under stimulated conditions was increased from 0.39 ± 0.05 to 0.88 ± 0.06 in the presence of DOC, suggesting that

Table 1 Effect of DOC on the stimulation of [^3H]InsP and [^3H]InsP₂ formation by GTP[S] and CCh in mouse cortical membranes

Labelled membranes (150 μg of protein) were incubated for 15 min in the absence (basal) or presence of 1 μM GTP[S] with or without 1 mM CCh. Reactions were stopped and [^3H]inositol phosphates separated by Dowex anion-exchange chromatography as described in the Materials and methods section. DOC (1 mM) was present where indicated. Results are expressed as means $\pm$ S.E. for one experiment performed in triplicate. Similar results were obtained in four more experiments. * Indicates significantly different from GTP[S] alone (Student's *t* test).

	Amount formed (d.p.m./mg of protein)	
	[^3H]InsP	[^3H]InsP ₂
DOC absent		
Basal	4767 $\pm$ 542	1887 $\pm$ 223
GTP[S]	11750 $\pm$ 773	16120 $\pm$ 1400
GTP[S] + CCh	14450 $\pm$ 612*	19290 $\pm$ 1260*
DOC present		
Basal	6733 $\pm$ 570	3720 $\pm$ 315
GTP[S]	22800 $\pm$ 1360	23060 $\pm$ 1533
GTP[S] + CCh	51500 $\pm$ 920*	48820 $\pm$ 300*

Figure 1 Effect of DOC on the time course of $[^3\text{H}]\text{InsP}$, $[^3\text{H}]\text{InsP}_2$ and $[^3\text{H}]\text{InsP}_3$ formation induced by GTP[S] and CCh in mouse cortical membranes

Labelled membranes (375 μg of protein) were incubated for 15 min at 37 °C without ($\circ$, $\bullet$) or with 1 μM GTP[S] plus 1 mM CCh (∇ , $\blacktriangledown$) in the presence (closed symbols) or absence (open symbols) of 1 mM DOC. Results are means $\pm$ range for two experiments performed in triplicate.

the detergent could be affecting the degradation of $[^3\text{H}]\text{Ins}(1,4)\text{P}_2$. We investigated this possibility by analysing the degradation of $[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ by non-labelled membranes (Table 4). The amount of $[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ degraded was 20% higher in the presence of DOC. Moreover, whereas in the absence of DOC the amount of initial radioactivity appearing in $[^3\text{H}]\text{InsP}_2$ was almost four times that recorded in $[^3\text{H}]\text{InsP}$, in the presence of DOC a similar amount of radioactivity appeared in both $[^3\text{H}]\text{inositol}$ phosphates. Therefore DOC appears to be enhancing $[^3\text{H}]\text{inositol}$ polyphosphate degradation, especially that of $[^3\text{H}]\text{InsP}_2$.

Interestingly, GTP[S] was more effective in inducing $[^3\text{H}]\text{Ins}4\text{P}$ and $[^3\text{H}]\text{Ins}(1,4)\text{P}_2$ accumulation in the absence (15.4-fold and 34.7-fold over basal respectively) than in the presence of DOC (4.4-fold and 8.5-fold). By contrast, CCh potentiation of the nucleotide effect was higher when DOC was present (1.3-fold without DOC and 2-fold with DOC, for both isomers) (Table 3). This suggested that the detergent was affecting the coupling of the components of the transduction system (receptor, G-protein, PIC). Therefore we determined the effect of DOC on the

Table 2 Effect of DOC on $[^3\text{H}]\text{phosphoinositide}$ breakdown induced by GTP[S] and CCh in mouse cortical membranes

Labelled membranes (150 μg of protein) were incubated for 15 min in the absence (basal) or presence of 1 μM GTP[S] with 1 mM CCh. DOC (1 mM) was present where indicated. Phospholipids were deacylated and water-soluble products separated by Dowex anion-exchange chromatography. Results are expressed as the percentage of radioactivity present at zero time and are means $\pm$ S.E.M. for seven experiments performed in triplicate. Incorporation of $[^3\text{H}]\text{inositol}$ into lipids at zero time was 606412 ± 132230 d.p.m./mg of protein and the relative proportions of $[^3\text{H}]\text{label}$ in PtdIns, PtdInsP and PtdInsP₂ were 92.6 ± 1.3 , 6.8 ± 1.1 and $0.7 \pm 0.2\%$ of total respectively. *Indicates significantly different from basal (paired Student's *t* test).

	Percentage of radioactivity at zero time		
	$[^3\text{H}]\text{PtdIns}$	$[^3\text{H}]\text{PtdInsP}$	$[^3\text{H}]\text{PtdInsP}_2$
DOC absent			
Basal	92.5 ± 6.8	163.3 ± 14.4	211.2 ± 29.0
GTP[S] + CCh	91.1 ± 8.0	$142.8 \pm 11.3^*$	$165.5 \pm 19.1^*$
DOC present			
Basal	80.9 ± 6.4	288.6 ± 19.6	191.2 ± 20.9
GTP[S] + CCh	$72.1 \pm 5.0^*$	$198.0 \pm 7.3^*$	$135.9 \pm 13.8^*$

Table 3 Stimulation of isomeric $[^3\text{H}]\text{inositol}$ phosphates formation by GTP[S] and CCh in mouse cortical membranes in the presence or absence of DOC

Labelled membranes (150 μg of protein) were incubated for 15 min at 37 °C in the absence (basal) or presence of 1 μM GTP[S] with or without CCh (1 mM). DOC (1 mM) was present where indicated. Reactions were stopped and $[^3\text{H}]\text{inositol}$ phosphates separated by h.p.l.c. as described in the Materials and methods section. Results are means $\pm$ S.E.M. for three experiments performed in triplicate. *,† Indicate significantly different from basal (*) or from GTP[S] alone (†) (paired Student's *t* test).

	Amount formed (d.p.m./mg of protein)		
	$[^3\text{H}]\text{Ins}1\text{P}$	$[^3\text{H}]\text{Ins}4\text{P}$	$[^3\text{H}]\text{Ins}(1,4)\text{P}_2$
DOC absent			
Basal	4342 ± 791	444 ± 182	511 ± 78
GTP[S]	4644 ± 835	$6375 \pm 884^*$	$17769 \pm 4571^*$
GTP[S] + CCh	4978 ± 922	$8495 \pm 711^{*\dagger}$	$22411 \pm 5049^{*\dagger}$
DOC present			
Basal	3422 ± 467	6035 ± 973	6384 ± 1560
GTP[S]	$5507 \pm 511^*$	$26778 \pm 2475^*$	$29295 \pm 5573^*$
GTP[S] + CCh	$8722 \pm 1102^*$	$47349 \pm 6513^{*\dagger}$	$54238 \pm 9615^{*\dagger}$

Table 4 Effect of DOC on the degradation of $[^3\text{H}]\text{inositol}$ phosphates by non-labelled mouse cortical membranes

$[^3\text{H}]\text{Ins}(1,4,5)\text{P}_3$ at 0.5 nM (14000 d.p.m.) was incubated for 15 min at 37 °C with non-labelled membranes (150 μg of protein) with or without 1 mM DOC. Reactions were stopped and $[^3\text{H}]\text{inositol}$ phosphates separated by Dowex anion-exchange chromatography. After 15 min incubation, 95% of the initial radioactivity was recovered as $[^3\text{H}]\text{inositol}$ phosphates under both conditions. Results are means $\pm$ range for two experiments performed in triplicate. * Indicates significantly different from results obtained in the absence of DOC (Student's *t* test).

DOC	$[^3\text{H}]\text{inositol}$ phosphates (% of total)		
	$[^3\text{H}]\text{InsP}$	$[^3\text{H}]\text{InsP}_2$	$[^3\text{H}]\text{InsP}_3$
Absent	16.7 ± 0.6	61.6 ± 0.1	21.6 ± 0.8
Present	$44.4 \pm 1.5^*$	$38.1 \pm 2.4^*$	$17.4 \pm 0.9^*$

Table 5 Parameters for stimulation of [^3H]InsP₂ formation by GTP[S] and CCh in mouse cortical membranes in the presence or absence of DOC

Labelled membranes (150 μg of protein) were incubated for 15 min at 37 °C with increasing concentrations of GTP[S] (0.01–100 μM) with or without 1 mM CCh. DOC (1 mM) was present where indicated. Concentration-effect curves were fitted to the Hill equation using a non-linear regression program. Results are means $\pm$ S.E.M. for three experiments performed in triplicate. Statistical analysis was performed using a paired Student's *t* test. * Indicates significantly different from GTP[S] alone, in the absence of DOC; † indicates significantly different from GTP[S] alone, in the presence of DOC; § indicates significantly different from GTP[S] with CCh, in the absence of DOC. E_{max} , maximal effect.

	EC_{50} (μM)	E_{max} (d.p.m./mg of protein)	Hill coefficient
DOC absent			
GTP[S]	0.025 $\pm$ 0.007	9967 $\pm$ 1693	0.85 $\pm$ 0.09
GTP[S] + CCh	0.014 $\pm$ 0.001	12220 $\pm$ 2000*	0.97 $\pm$ 0.19
DOC present			
GTP[S]	0.522 $\pm$ 0.210	21473 $\pm$ 3360*	0.76 $\pm$ 0.05
GTP[S] + CCh	0.070 $\pm$ 0.028	35567 $\pm$ 6620†§	0.82 $\pm$ 0.09

concentration-effect parameters for GTP[S] alone or in the presence of 1 mM CCh. As shown in Table 5 for [^3H]InsP₂ formation, (similar results were obtained for [^3H]InsP, not shown), the guanine nucleotide was 20-fold more potent in the absence of the detergent. In both cases, the agonist increased the potency of GTP[S], but much more in the presence (7.5-fold) than in the absence of DOC (1.8-fold). Moreover, the detergent produced a 2-fold increase in the efficacy of GTP[S] alone and a 3-fold increase when the agonist was present.

DISCUSSION

In the present study we have examined phosphoinositide breakdown in mouse brain cortical membranes directly labelled with [^3H]inositol in the presence of CMP. Under these conditions, [^3H]inositol incorporation into polyphosphoinositides occurs largely through the reverse PtdIns synthase reaction, as previously described [10,12]. When this method was used, levels of radioactivity incorporated into the phosphoinositide pool were higher than those obtained with the method (prelabelling cortical slices with [^3H]inositol [8]) used previously. Moreover, unlike in membranes from prelabeled slices, where agonist potentiation of GTP[S]-stimulated phosphoinositide breakdown is only observed in the presence of the anionic detergent DOC [8], in membranes labelled directly it was possible to observe a small, but significant, potentiation by CCh of the GTP[S] effect without adding DOC to the assay medium. This opened up the possibility of investigating how the detergent was affecting GTP[S]-dependent CCh stimulation of phosphoinositide breakdown.

The results of our study indicate that DOC is affecting the muscarinic receptor–G protein–PIC transduction system in several ways. Only in the presence of DOC was there an agonist-induced [^3H]PtdIns decrease. Results from HPLC analysis of [^3H]inositol phosphate isomers showed that a small, but significant, GTP[S] and agonist-induced [^3H]Ins1P accumulation over basal levels occurred only when DOC was present. In whole cells, this isomer could originate from PIC hydrolysis of PtdIns or from degradation of either Ins(1,3,4)P₃ [14] or Ins(1,4)P₂ [15]. However, Ins(1,3,4)P₃ originates from Ins(1,3,4,5)P₄, whose formation requires the action of Ins(1,4,5)P₃ 3-kinase, an enzyme that is absent in membranes [16]. Additionally, degradation of Ins(1,4)P₂ to [^3H]Ins1P has not been observed in broken-cell

preparations [15]. Thus the source of [^3H]Ins1P in our membrane system is probably [^3H]PtdIns. PIC hydrolysis of exogenous [^3H]PtdIns in a DOC-containing assay system has been also reported by Claro et al. [17]. In the absence of DOC, there was no significant formation of [^3H]Ins1P, indicating that, in intact membranes, as in brain slices [15], [^3H]PtdIns is not a substrate of receptor–G-protein-activated PIC.

Most of the agonist-induced decrease in [^3H]PtdIns levels observed in the presence of the detergent appears to result from its conversion into [^3H]PtdInsP. As shown in Table 2, under basal conditions, DOC greatly increases the formation of [^3H]PtdInsP, indicating that the detergent is activating PtdIns 4-kinase. Activation by detergents of different isoforms of PtdIns 4-kinase purified from rat brain and other tissues has been observed previously [18]. Conversely, PtdInsP 5-kinase does not seem to be significantly affected, since [^3H]PtdInsP₂ levels are similar in the presence and absence of DOC.

Both in the absence and presence of DOC, [^3H]Ins4P and [^3H]Ins(1,4)P₂ accounted for most of the [^3H]inositol phosphates formed (Table 3). These isomers can result from the degradation of the PIC hydrolysis products of [^3H]PtdInsP₂ and/or [^3H]PtdInsP. Accordingly, there was a higher relative decrease of these polyphosphoinositides compared with [^3H]PtdIns. In brain slices, both [^3H]PtdInsP₂ and [^3H]PtdInsP appear to be substrates for CCh-stimulated PIC [15]. In CCh-stimulated membranes (Figure 1), the initial rate of [^3H]InsP₂ formation (1500 and 3950 d.p.m./min respectively) was higher than that of [^3H]InsP₃ (410 and 570 d.p.m./min) in both the absence and presence of DOC. Rates of [^3H]InsP₃ formation will be underestimated, owing to its rapid degradation by the membrane-bound InsP₃ 5-phosphatase, and more so in the presence of DOC (Table 4). However, the high formation rate, together with the high levels of [^3H]InsP₂ obtained, suggest that both [^3H]PtdInsP₂ and [^3H]PtdInsP are substrates for PIC. That is more apparent in the presence of DOC, where the initial rate of [^3H]InsP₂ accumulation was 2.6-fold higher than in the absence of the detergent. This may result from the higher levels of [^3H]PtdInsP present in the presence of DOC (Table 2). Moreover, it is also possible that DOC changes the preference of PIC for [^3H]PtdInsP over [^3H]PtdInsP₂, since the percentage decrease in [^3H]PtdInsP levels with respect to that of the total [^3H]polyphosphoinositides is significantly higher (51.4%) than that observed without DOC (36.2%). Our results also indicate that the detergent enhances agonist-induced PIC activity towards [^3H]PtdInsP₂, because the percentage decrease was significantly larger than in the absence of the detergent, and steady-state levels of [^3H]InsP₃ were doubled. In agreement with this, it has been reported that DOC enhances the activity of purified PIC [19,20].

Apart from increasing the stimulated formation of [^3H]Ins(1,4)P₂ and [^3H]Ins4P, DOC affected the relative proportion of these two isomers (Table 3). The higher increase in [^3H]Ins4P compared with [^3H]Ins(1,4)P₂ appears to be due to an enhancement of Ins(1,4)P₂ degradation by the detergent. As shown in Table 4, when degradation of exogenous [^3H]Ins(1,4,5)P₃ in unlabelled membranes was examined the detergent significantly enhanced the degradation of [^3H]InsP₂ and to a lesser extent that of [^3H]InsP₃. In brain, the Ins(1,4)P₂ 1-phosphatase is a partly membrane-bound enzyme [21] that may be activated by detergents as it has been reported for Ins(1,4,5)P₃ 5-phosphatase [22].

Finally, the results in Table 5 show that DOC decreases the affinity of the G-protein for GTP[S] (20-fold decrease in potency). Conversely, a similar potency was observed in the presence of the agonist. Thus, in the presence of DOC, the agonist induces a larger leftward shift in the GTP[S] dose-response curve, making

more evident the agonist activation of the G-protein. Blank et al. [23] described an uncoupling effect of cholate on the G_q -protein-PIC system in bovine liver membranes. Since, in our membrane preparation, the G-protein appears to have a diminished ability to be regulated by the muscarinic receptor, the possibility exists that an endogenous factor which acts like DOC to allow receptor regulation of G-protein activity may have been lost in the course of tissue homogenization and membrane preparation. This point merits further investigation.

In the light of the array of alterations observed here in the enzymic steps of phosphoinositide cycle and the coupling of the muscarinic receptor-G-protein-PIC system when membranes are stimulated in the presence of DOC, caution is advised when interpreting results concerning PIC substrate specificity and the potency of both guanine nucleotides and agonists from experiments performed in the presence of DOC or related bile salts.

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