Biochemical Pharmacology, Vol. 51, pp. 77–82, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02129-9

Modulation of Serotonin Binding Sites in Spisula Solidissima Oocytes by Phorbol Ester

*Pierre Rivailler and Slavica Krantic**

ECOLE NORMALE SUPÉRIEURE, LABORATOIRE DE BIOLOGIE MOLÉCULAIRE ET CELLULAIRE, 46 ALLÉ D'ITALIE, 69364 LYON CÉDEX 07, FRANCE

ABSTRACT. In *Spisula solidissima* oocytes, serotonin (5-hydroxytryptamine, 5-HT)-dependent meiosis reinitiation is mediated via specific 5-HT membrane binding sites. This oocyte response is inhibited by the phorbol ester TPA. To assess whether the inhibitory effect of TPA was due to alteration of oocyte membrane binding sites, we studied their characteristics after TPA treatment. [^{3}H]-5HT binding assays revealed that TPA decreased the affinity and, after prolonged treatment, increased the number of oocyte binding sites. Moreover, inhibitory actions of TPA on 5-HT-induced meiosis reinitiation paralleled its inhibitory effects on 5-HT binding site affinity. The inhibitory actions in biological assays were restricted to TPA (an inactive analog of TPA, TPA-met was inefficient) and were completely reversed by staurosporine. Our data thus suggest an inhibitory role for protein kinase C on oocyte 5-HT binding sites under physiological conditions. BIOCHEM PHARMACOL 51;1:77–82, 1996.

KEY WORDS. serotonin binding site; phorbol ester; oocyte; clam; meiosis reinitiation; protein kinase C

Surf clam *Spisula solidissima* oocytes, naturally arrested during prophase of first maturation division, reinitiate meiosis after fertilization or application of chemical stimuli such as KCl [1] or phorbol esters [2]. The first visible response of oocytes to activation consists of GVBD†[2], followed by chromosome condensation, extrusion of the first and second polar bodies, and pronuclei formation. It has also been shown that the bivalve mollusc neurohormone, serotonin, 5-hydroxytryptamine-5-HT [3, 4], could induce GVBD and polar body extrusion in the surf clam [5]. These biological effects of serotonin are mediated by an oocyte membrane 5-HT binding site with unique pharmacological profile and ligand binding characteristics that has been named 5-HT_5 [6]. It has been proposed that such a 5-HT_5 binding site plays the role of physiological receptor by mediating 5-HT effects in oocytes, since its pharmacological profile [6] was almost identical with the pharmacological profile of 5-HT-induced GVBD [7]. Simultaneously, two other 5-HT receptors were cloned from mouse brain cDNA library and named 5-$HT_{5A\&B}$ [8]. To avoid confusion, in the rest of this paper, clam receptor will be designated as *Spisula* oocyte 5-HT binding site.

Few additional invertebrate 5-HT receptors have been previously identified by molecular cloning: Three are different subtypes in *Drosophila* [9, 10] and one, distinct from the *Drosophila* receptor, is a subtype in *Lymnaea* [11]. All of these, together with the majority (5-HT_{1A-F}, 5-HT_{2A-C}, 5-HT_{4-7}) of mammalian 5-HT receptor groups (5-HT_{1-7}), belong to the supergene family of G protein-linked receptors (5-HT_3 receptor is not a member of this family—it is a ligand-gated ion channel [12]). The primary structures and mechanisms of regulation have been elucidated for a number of mammalian receptors [12, 13, and references therein]. However, the regulation of invertebrate 5-HT receptors is still poorly understood. In particular, the possible involvement of protein kinase C (PKC) in the regulation of invertebrate 5-HT receptors has not been assessed as yet.

An activator of PKC such as the phorbol ester [14] TPA (12-O-tetradecanoyl-13-phorbol acetate) inhibits *Spisula* oocyte response to 5-HT; it triggers concomitantly an increase in IC_{50} value (5-HT concentration necessary to induce 50% GVBD) and a decrease in maximal oocyte response to 5-HT [7]. This functional antagonism between 5-HT and TPA effects might be caused by the inhibitory effects of PKC on the *Spisula* 5-HT binding site mediating 5-HT-induced GVBD [7]. Negative feedback of PKC at the receptor level has already been reported for mammalian growth factor receptors [15]. To test whether the inhibitory effects of TPA on 5-HT-induced meiosis reinitiation involved a similar negative effect on receptor function, we studied the kinetic parameters of *Spisula* 5-HT binding sites after different time exposures of oocytes to TPA.

* Corresponding author. Tel. 33-72-72-80-13; FAX 33-72-72-86-86.

† Abbreviations: GVBD, germinal vesicle breakdown; 5-HT, 5-hydroxytryptamine; TPA, 12-O-tetradecanoyl-13-phorbol acetate; TPA-met, phorbol-12-myristate-13-acetate-4-O-methyl-ether; LSD, lysergic acid diethylamide; PKC, protein kinase C.

Received 8 October 1994; accepted 11 September 1995.

MATERIAL AND METHODS

Handling and Treatment of Oocytes

Surf clams were collected at Iles de la Madeleine (Québec, Canada). Oocytes were obtained and handled according to Allen [1]. Oocyte suspensions (obtained from 2–4 females), adjusted to 0.5% concentration in artificial sea water (ASW), were either incubated with supramaximal concentration (200 nM) of TPA alone [7], or simultaneously with TPA (200 nM) and 5-HT (5 μM) [7], or left untreated (control group). In some experiments, oocytes were treated with 1 μM of an inactive TPA analog, TPA-met (phorbol-12-myristate-13-acetate-4-O-methyl-ether) alone or in the presence of 5 μM 5-HT. Oocyte responses were quantified by scoring GVBD as previously reported [7].

Preparation of Oocyte Membrane and/or Vitelline Coat Fractions

Each batch of oocytes used for membrane and/or vitelline coat fraction preparation consisted of gametes obtained from 4–6 females; it was initially divided into 5 groups. Each group was then exposed to TPA (200 nm) for different time periods (0, 15, 30, 45, and 90 minutes). After washing in ASW, oocyte plasma membrane and vitelline coat fractions were obtained as previously described [6]. Briefly, the vitelline coat of the oocyte was removed by incubation with 1 M glycerol in 20 mM phosphate buffer, pH 8 (1:10, vol/vol), for 3 min at 25°C. The oocyte suspension was sedimented by quick centrifugation (Tabletop International Centrifuge, setting no. 2, 2 min) to separate supernatant (containing vitelline envelopes) and pellet (containing vitelline envelope-free oocytes). Oocyte membrane and vitelline coat fractions were then prepared from pellet and supernatant, respectively, by differential centrifugation as detailed elsewhere [6].

Binding Assays

Binding assays were performed by incubating either fraction (protein equivalent: 40–80 μM per 100 μL) with 100 μL of [^{3}H]-5HT (concentration range from 0.08 μM to 1 μM for control group and 0.08 μM to 1.5 μM for TPA-treated groups) in the final volume of 1.5 mL of 50 mM Tris-HCl buffer, pH 7.4 for 30 min at 25°C (for details, see ref. [6]). The nonspecific (NS) component of total (T) binding was determined in the presence of 100 μM nonradioactive 5-HT. The incubation was stopped by filtration over Whatman GF/B glass fiber filters (presoaked in 0.1% polyethylenamine for 2 hr) at weak pressure. Filters were rapidly rinsed three times with 5 mL of ice-cold Tris-HCl buffer. This step was accomplished in less than 15 sec for each individual experimental point in order to minimize [^{3}H]-5HT dissociation from the complex [^{3}H]-5HT/binding site. Filters (placed in 7 mL of Aquasol, NEN, France) were assayed for radioactivity by liquid scintillation counting with 60% efficiency.

Data Analysis

Binding data were expressed in picomoles of [^{3}H]-5HT bound per mg of protein. Protein concentration was measured according to Lowry and colleagues [16]. Kinetic parameters (dissociation constant, K_d, and maximal binding capacity, B_{max}) were evaluated by computer-assisted nonlinear regression analysis using McPherson's modified Ligand program [17].

Differences between percentage of GVBD, on the one hand, and binding parameters, on the other, determined for control and TPA-treated groups were statistically assessed using one-way analysis of variance (ANOVA) and considered significant at $P < 0.05$.

RESULTS

Kinetic Characteristics of 5-HT Receptors of TPA-Treated Spisula solidissima *Oocytes*

For the control, the specific component of [^{3}H]-5HT binding was saturable. Scatchard transformation of equilibrium binding data yielded a single slope plot with a dissociation constant (K_d) of 0.35 ± 0.08 μM (mean ± SE, $n = 6$) and a maximal binding capacity (B_{max}) of 2.72 ± 0.52 pmol/mg protein (Figs. 1A and 2A,B). These experiments were repeated six times using membrane preparations obtained from six different batches of oocytes. In each individual experiment, triplicate determinations of [^{3}H]-5HT binding were performed with 9 increasing concentrations of the radioligand for both total and nonspecific components of binding.

After treatment with TPA (200 nM) for 90 min, [^{3}H]-5HT specific binding displayed a tendency to saturation, but was not completely saturated even at the highest [^{3}H]-5HT concentration (1.5 μM) tested. K_d value estimated under these conditions was increased 4–6 fold (1.67 ± 0.25 μM; mean ± SE, $n = 4$) above the control group (Figs 1B and 2A). Corresponding B_{max} values were also increased in this group as compared to the controls (Figs. 1B and 2B). The latter results were obtained in four independent experiments on oocyte membrane preparations obtained from four different batches of TPA-treated oocytes. In each of these experiments, 12 concentrations of [^{3}H]-5HT were used for triplicate determinations of both total and nonspecific [^{3}H]-5HT binding to membrane preparations.

To determine the minimal time exposure of oocytes to TPA sufficient to induce a significant decrease in *Spisula* 5-HT oocyte binding site affinity, we then examined their kinetic characteristics after 15, 30, and 45 min of TPA treatment. The apparent affinity of the *Spisula* 5-HT binding site was significantly lowered after TPA treatment of at least 30 min (Fig. 2A), whereas a significant increase in the B_{max} value was not observed up to 90 min (Fig. 2B).

In contrast, 5-HT binding sites of vitelline coat fractions (prepared from the same oocyte batches used for membrane fraction preparation; see *Material and Methods*) were apparently unaffected by TPA treatment. K_d were 1.31 ± 0.10 μM (mean ± SE, $n = 6$) and 1.70 ± 0.20 μM (mean ± SE, $n = 4$), while B_{max} were 0.199 ± 0.020 pmol/mg protein (mean ± SE,

$n = 6$) and 0.156 ± 0.064 pmol/mg protein (mean ± SE, $n = 4$) in control and 90-min TPA treated groups, respectively.

Nonlinear regression analysis [17] of experimental versus computed data for experimental points showed that they fit the one-site receptor model better ($P < 0.05$) than the two-site model.

Correlation of TPA Effects in Binding Assays to Its Effects in Biological Assays

$GVBD_{50}$ (time when 50% oocytes were activated), which normally occurs within 8 to 10 min after 5-HT treatment, took place after more than 30 min when TPA and 5-HT were added simultaneously (Fig. 3). In contrast, an inactive TPA analog, TPA-met, had no effect on *Spisula* oocytes when added alone (data not shown), and did not alter the kinetics of 5-HT-induced GVBD when added simultaneously with the neurohormone (Fig. 3). If given enough time (30 min and 90 min, respectively), oocyte groups treated with either 5-HT alone or simultaneously with 5-HT and TPA achieved 100% GVBD. However, an exposure of more than 90 min to TPA (the

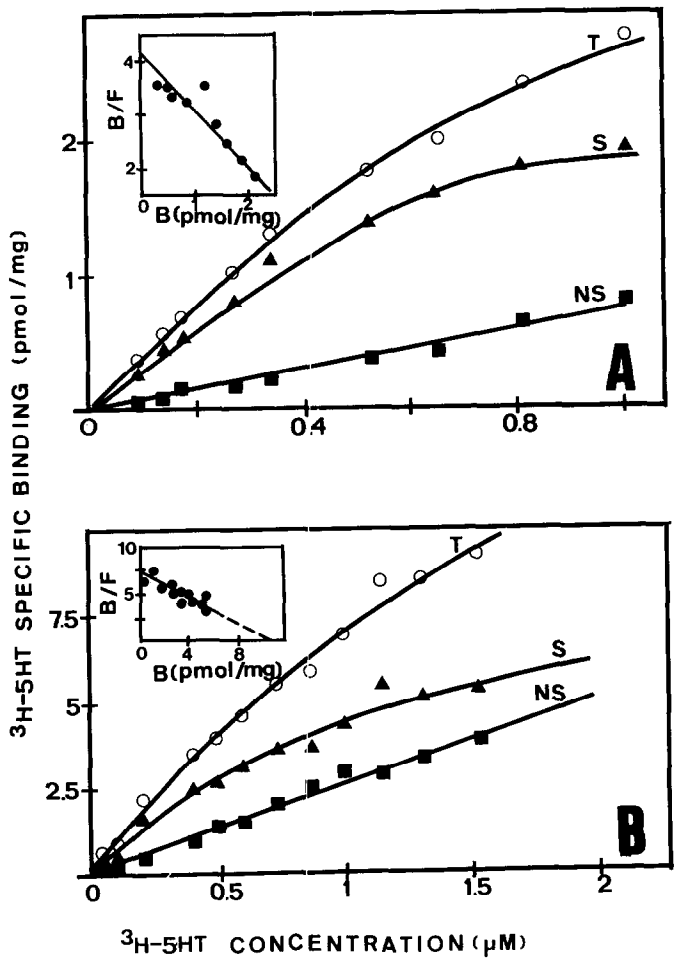

FIG. 1. Saturation of [^{3}H]-5-HT specific binding by increasing free [^{3}H]-5-HT concentrations in membrane preparation obtained from one batch of oocytes. Prior to membrane preparations (see Materials and Methods), the batch was divided into two parts: incubated either in absence (A) or presence (B) of 200 nM TPA for 90 min. Insets represent the Scatchard linearization of specific binding data obtained in this representative experiment. Each value is the mean (SE were less than 5%) of triplicate determinations of [^{3}H]-5-HT binding for a given free [^{3}H]-5HT concentration performed either in absence (T = "total binding") or in presence (NS = "nonspecific binding") of 100 μM of non-radioactive 5-HT. "Specific" (S) binding was determined as the difference between "total" and "nonspecific" binding.

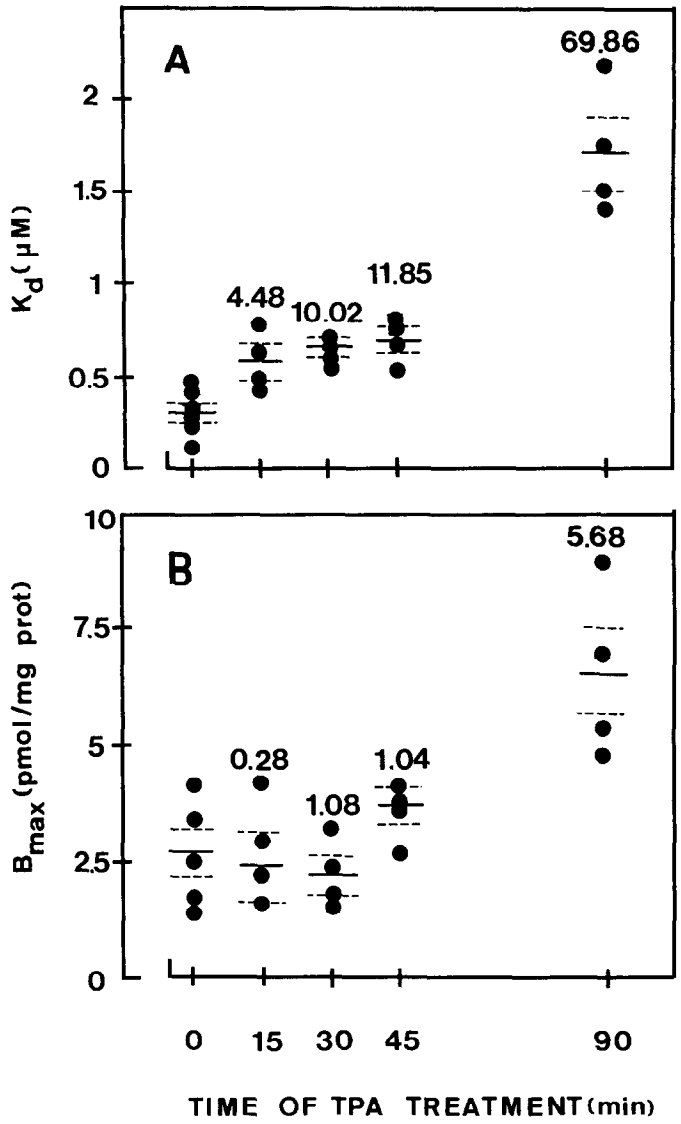

FIG. 2. Individual K_d (A) and B_{max} (B) values estimated for [^{3}H]-5-HT specific binding after different periods of exposure to TPA. Each point represents a value determined in at least four independent experiments (similar to those presented in Fig. 1) performed on membrane preparations obtained from different batches of oocytes (n = 4–6). Each batch of oocytes was initially divided into five parts, which were either left untreated and immediately used for membrane preparations ("0" time point) or first treated with 200 nm TPA for 15, 30, 45, and 90 min, then washed and subsequently used for preparation of membrane fractions and binding assays (see Materials and Methods). For both K_d and B_{max} parameters, mean value is given by a solid line, SE limit by a dashed line. Numbers represent calculated F values (at significance limit $P = 0.05$, table F value is 5.59).

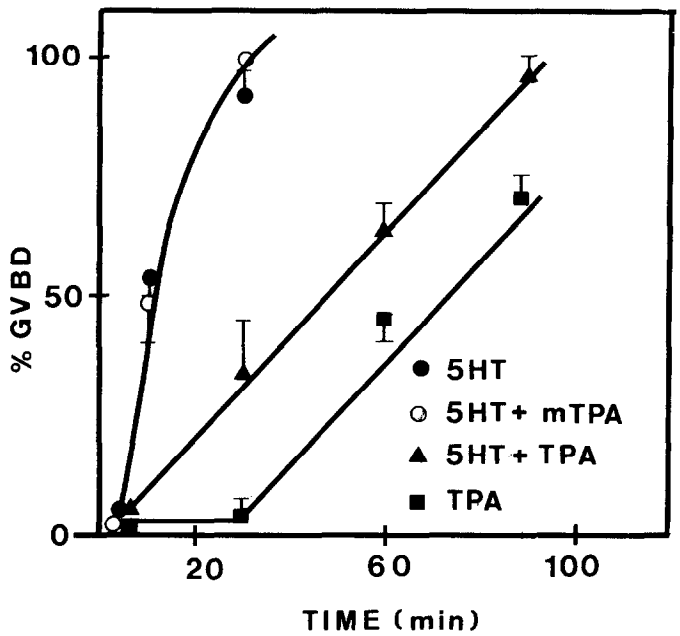

FIG. 3. Kinetics of oocyte activation and the effect of TPA on these kinetics. In three independent experiments, the oocyte suspensions from three batches (each obtained by gonad dissection from 2–4 females) were divided into four parts: Two parts were incubated with either TPA (200 nM) or 5-HT (5 μM) alone while the other two were incubated simultaneously with 5-HT (5 μM) and either TPA (200 nM) or TPA-met (1 μM). The GVBD was then scored after indicated time periods as previously described [7]. Each value represents the mean ± SE (n = 3).

longest time period tested) was necessary to obtain 100% of GVBD, since only 85 ± 5% of oocytes treated with TPA alone displayed GVBD at that time point (Fig. 3).

In order to check whether the observed inhibitory actions of TPA on 5-HT-induced GVBD involve PKC, we studied the effects of staurosporine, a relatively specific PKC inhibitor [18]. Staurosporine completely inhibited TPA-induced GVBD in a dose-dependent manner with an IC_{50} value of 0.43 ± 0.12 μM (Fig. 4; this was used as a positive control). In contrast, staurosporine reversed the inhibition exerted by TPA on 5-HT-induced GVBD with an IC_{50} concentration of 0.45 ± 0.14 μM (Fig. 4). The observed effects of staurosporine could not be attributed to its antibiotic actions, since other antibiotics (penicillin, gentamycin) had no effect on GVBD under either experimental condition (data not shown).

DISCUSSION

The present study used radioligand binding assay on membrane homogenates to characterize the effect of TPA on *Spisula* oocyte 5-HT binding sites. The kinetic characteristics of the plasma membrane 5-HT binding sites, evaluated from the control group, is consistent with previously reported data [6].

Our results imply that TPA triggers a significant decrease in *Spisula* 5-HT oocyte binding site affinity and, at least after 90-min TPA treatment, an increase in binding site concentration. This is to our knowledge the first report on the regulation of invertebrate 5-HT binding sites by TPA. However, an unequivocal demonstration of such a regulation must await the discovery of ligands with nanomolar affinity for the *Spisula* oocyte 5-HT binding site. Indeed, the relatively low affinity of this receptor for [^{3}H]-5HT (0.2–0.4 μM) does not permit the accurate determination of its kinetic parameters (K_d and B_{max}) under experimental conditions such as TPA treatment which further decreases its affinity. Consequently, optimal assay conditions could not be achieved in experiments performed on membranes obtained from oocytes treated with TPA for 90 min (as illustrated in Fig. 1B), since the nonspecific binding component of total binding was excessive at [^{3}H]-5HT concentrations higher than the K_d value. However, [^{3}H]-LSD, used as a ligand of choice for characterization of some mollusc 5-HT receptors displaying nanomolar affinity for this ligand [11], is not advantageous in our model system. Indeed, ergot derivatives structurally related to LSD display low (micromolar) affinity for 5-HT oocyte receptors of *Spisula* [7] and of another, closely-related bivalve species [19]. However, keeping this technical limitation in mind, it seems reasonable to assume that TPA alters the characteristics of the *Spisula* oocyte 5-HT binding site, since a decrease in receptor affinity (but not in receptor concentration) was detected after only 30 min of TPA treatment; the highest K_d value determined in this experimental group was 0.71 μM (see Fig. 2A) for the highest free [^{3}H]-5HT concentration of 1.5 μM. Similarly, the deter-

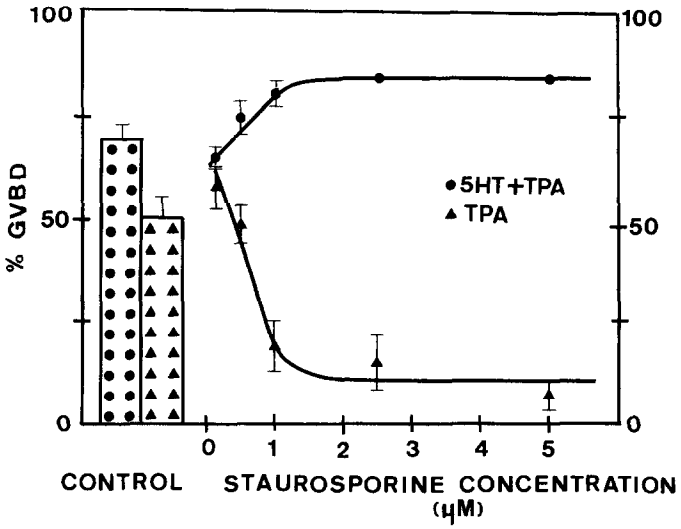

FIG. 4. Effects of staurosporine on TPA-mediated inhibition of 5-HT-induced GVBD. The same batch of oocytes was initially divided into two parts; one part was incubated without staurosporine (depicted as "control" histograms), and the other was subdivided and then incubated with indicated staurosporine concentrations for 16 hours. During the last 60 min of incubation, either TPA (200 nM) alone or TPA (200 nM) and 5-HT (5 μM) were added to oocyte suspensions. The GVBD was then scored as described [7]. Each point on the dose-response curves represent the mean ± SE of data obtained in three independent experiments performed in duplicates, using three different batches of oocytes.

mination of binding site affinity and concentration in the 45-min treatment group is reliable, since the highest [^{3}H]-5HT concentration used represents at least a two-fold K_d value. Therefore, only K_d and B_{max} estimates for the 90-min TPA-treated group should be viewed with caution.

Conversely, TPA treatment had no effect on vitelline coat 5-HT binding sites. Therefore, these binding sites appear to be regulated in a different way from the regulation of plasma membrane receptors. Intracellular location of the TPA target, PKC, might be the cause of such a lack of effect. Indeed, PKC activity has never been reported in vitelline coat fractions. Moreover, PKC is obviously activated at the site of diacylglycerol production (i.e. at the cytoplasmic side of plasma membrane), and would not be secreted out of cell in vitelline coat.

Inhibitory effects of TPA on 5-HT binding site affinity of *Spisula* oocyte paralleled inhibitory actions of TPA on 5-HT-induced GVBD, thus suggesting a role for PKC in regulating the receptor function in physiological conditions. This conclusion was further strengthened by our data concerning staurosporine effects on TPA-mediated inhibition of 5-HT-induced meiosis reinitiation. This alkaloid was initially reported as a highly selective and potent inhibitor of PKC [18]. However, later studies [20, 21] have shown that staurosporine, like other PKC inhibitors such as H-7, sphingosine, W-7, palmitoylcarnitine, and chlorpromazine [22–24] displays only a limited selectivity towards PKC; staurosporine also inhibits cyclic nucleotide-dependent kinases and protein tyrosine kinases. We chose staurosporine because of its relatively high potency [23] and its noncompetitive inhibition of PKC activation by TPA, the latter characteristic making it possible to neglect a difference in respective affinities of TPA and staurosporine for PKC binding [18, 20].

Under our experimental conditions, staurosporine completely inhibited TPA-induced oocyte activation, thus strongly suggesting that in the range of the inhibitor concentrations used, PKC was efficiently inhibited. Indeed, it is generally accepted that TPA targets PKC specifically [14]. Determined IC_{50} concentration for staurosporine effects was in accord with the concentration range previously reported to correspond to the specific actions of staurosporine on PKC *in vivo* [see ref. 22 and references therein]. In addition, the IC_{50} value obtained for the staurosporine effect on TPA-dependent inhibition of 5-HT-induced GVBD was identical to the IC_{50} value obtained for the staurosporine effect on TPA-induced GVBD, thus strongly suggesting that the same effector (i.e. PKC) mediates both oocyte responses. Likewise, a similar inhibition of oocyte response to 5-HT has been reported in *Xenopus* oocytes expressing an exogenous 5-HT receptor (5-HT_{1C}). Indeed, the TPA-dependent activation of PKC reduced the 5-HT-triggered Cl^- current across *Xenopus* oocyte plasma membrane [25–27].

What is the mechanism of TPA-mediated inhibition of 5-HT-induced GVBD in *Spisula* oocytes? It can be hypothesized that PKC decreases *Spisula* oocyte 5-HT binding site affinity by phosphorylating the sites. Indeed, Eckberg and collaborators have reported the functional involvement of PKC in regulating GVBD in *Spisula solidissima* oocytes [24]. This might be relevant to the data reported on physiological regulation of some G-protein coupled receptors by PKC phosphorylation. For example, muscarinic cholinergic receptor desensitization is mediated by PKC phosphorylation [28]. Similarly, it has been shown that TPA inhibition of 5-HT_{1A} receptor coupling to adenylyl cyclase involved phosphorylation of 5-HT_{1A} [29] on a PKC consensus site [30]. The consensus site for PKC phosphorylation has been identified in most cloned 5-HT receptors; it is located in the third cytoplasmic loop [30, 31]. This site of phosphorylation is very close to the cytoplasmic receptor domain that interacts with G-proteins [31].

Although coupling of the *Spisula* oocyte 5-HT binding site has not been directly characterized, our preliminary results pointed to GTP-sensitive binding of [^{3}H]-5HT to the membrane receptor sites (data not shown), thus indicating their coupling to "G" protein. These data, together with recently published results on facilitation of 5-HT-induced GVBD by G-protein activators (mastoporan and mas-7) in a closely-related clam species [32], add further arguments to the hypothesis concerning the possible inhibition of *Spisula* oocyte 5-HT receptor by PKC phosphorylation.

In conclusion, the inhibitory effects of TPA on *Spisula* oocyte response to 5-HT are, at least in part, accounted for by the actions of this phorbol ester on efficiency of transduction *via* the oocyte 5-HT binding site. Indeed, TPA decreases specifically (in comparison to vitelline coat binding sites) the oocyte plasma membrane receptor affinity. However, after the prolonged treatment, an apparent compensatory increase in plasma membrane receptor concentration was observed. Therefore, although very different from vertebrate 5-HT receptors [see ref. 6 and 7], invertebrate receptors such as *Spisula* 5-HT oocyte receptors, at least in some instances, might be regulated in a similar manner.

This study was partly realised in the laboratory of Dr. François Dubé (UQAR, Rimouski, Québec). We are grateful for his hospitality, interest and support. We also thank Glynis Thoiron for English revision.

References

1. Allen RD, Fertilization and artificial activation in the egg of the surf clam *Spisula solidissima*. *Biol Bull* **105:** 213–239, 1953.
2. Dubé F, Golsteyn R and Dufresne L, Protein kinase C and meiotic maturation of surf clam oocytes. *Biochem Biophys Res Comm* **142:** 1072–1076, 1987.
3. Matsutani T and Nomura T, Serotonin-like immunoreactivity in the central nervous system and gonad of the scallop, *Patinopecten yessoensis*. *Cell Tissue Res* **244:** 515–517, 1986.
4. Vitellaro-Zuccarello L, De Biasi S, Bernardi P and Oggioni A, Distribution of serotonin-, and gamma-aminobutyric acid- and substance P-like immunoreactivity in the central and peripheral nervous system of *Mytilus galloprovincialis*. *Tissue and Cell* **23:** 261–270, 1991.
5. Hirai S, Kishimoto T, Kadam AL, Kanatani H and Koide SS, Induction of spawning and oocyte maturation by 5-hydroxytryptamine in the surf clam. *J Exp Zool* **245:** 318–321, 1988.
6. Krantic S, Guerrier P and Dubé F, Meiosis reinitiation in surf clam oocytes is mediated via a 5-hydroxytryptamine$_5$ serotonin membrane receptor and a vitelline envelope-associated high affinity binding site. *J Biol Chem* **268:** 7983–7989, 1993.

7. Krantic S, Dubé F, Quirion R and Guerrier P, Pharmacology of the serotonin-induced meiosis reinitiation in *Spisula solidissima* oocytes. *Dev Biol* **146:** 491–498, 1991.
8. Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat J-L, Muscatelli F, Mattei M-G and Hen R, Mouse 5-hydroxytryptamine 5B receptors define a new family of serotonin receptors: Cloning, functional expression and chromosomal localization. *Mol Pharmacol* **43:** 313–319, 1993.
9. Witz P, Amlaiky N, Plassat J-L, Maroteaux L, Borreli E and Hen R, Cloning and characterization of a *Drosophila* serotonin receptor that activates adenylate cyclase. *Proc Natl Acad Sci USA* **87:** 8940–8944, 1990.
10. Saudou F, Boschert U, Amlaiky N, Plassat J-L and Hen R, A family of *Drosophila* serotonin receptors with distinct intracellular signaling properties and expression patterns. *EMBO J* **11:** 7–17, 1992.
11. Sugamori KM, Sunahara RK, Guan HC, Bulloch AGM, Tensen CP, Seeman P, Niznik HB and Van Tol HHM, Serotonin receptor cDNA cloned from *Lymnaea stagnalis*. *Proc Natl Acad Sci USA* **90:** 11–15, 1993.
12. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharne EJ, Saxena PR and Humphrey PPA, VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* **46:** 157–203, 1994.
13. Shen Y, Monsma FJ, Metcalf MA, Jose PA, Hamblin MW and Sibley DR, Molecular cloning and expression of 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J Biol Chem* **268:** 18200–18204, 1993.
14. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U and Nishizuka Y, Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257:** 7847–7851, 1982.
15. Nishizuka Y, Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258:** 607–614, 1992.
16. Lowry OH, Rosebrough NJ, Farr AL and Randall R, Protein measurement with folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
17. McPherson GA, A practical computer-based approach to the analysis of radioligand binding experiments. *Comput Prog Biomed* **17:** 107–114, 1983.
18. Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M and Tomita F, Staurosporine, a potent inhibitor of phospholipid/Ca^{++} dependent protein kinase. *Biochem Biophys Res Comm* **135:** 397–402, 1986.
19. Matsutani T and Nomura T, *In vitro* effects of serotonin and prostaglandins on release of eggs from the ovary of the scallop, *Patinopecten yessoensis*. *Gen Comp Endocrinol* **67:** 111–118, 1987.
20. Nakano H, Kobayashi E, Takahashi I, Tamaoki T, Kuzuu Y and Iba H, Staurosporine inhibits tyrosine-specific protein kinase activity of Raous sarcoma virus transforming protein p60. *J Antibiot* **40:** 706–708, 1987.
21. Tischler AS, Ruzicka LA and Perlman RL, Mimicy and inhibition of nerve growth factor effects: Interactions of staurosporine, forskolin and K252a in PC12 cells and normal chromaffin cells in vitro. *J Neurochem* **55:** 1159–1165, 1990.
22. Sako T, Tauber AI, Jeng AY, Yuspa SH and Blumberg PM, Contrasting actions of staurosporine, a potent protein kinase C inhibitor, on human neutrophils and primary mouse epidermal cells. *Cancer Res* **48:** 4646–4650, 1988.
23. Rueg UT and Burgess GM, Staurosporine, K252 and UCN-01: Potent but nonspecific inhibitors of protein kinases. *Trends Pharmacol Sci* **10:** 218–220, 1989.
24. Eckberg WR, Szuts EZ and Carroll AG, Protein kinase C activity, protein phosphorylation and germinal vesicle breakdown in *Spisula* oocytes. *Dev Biol* **124:** 57–64, 1987.
25. Ito I, Hirono C, Yamagishi S, Nomura Y, Kaneko S, Sugiyama H, Roles of protein kinases in neurotransmitter responses in *Xenopus* oocytes injected with rat brain RNA. *J Cell Physiol* **134:** 155–160, 1988.
26. Kato K, Kaneko S and Nomura Y, Phorbol ester inhibition of current responses and simultaneous protein phosphorylation in *Xenopus* oocyte injected with brain mRNA. *J Neurochem* **50:** 766–773, 1988.
27. Moran O and Dascal N, Protein kinase C modulates neurotransmitter responses in *Xenopus* oocytes injected with rat brain RNA. *Mol Brain Res* **5:** 193–202, 1989.
28. Richardson RM, Ptasienski J and Hosey MM, Functional effects of protein kinase C-mediated phosphorylation of chick heart muscarinic cholinergic receptors. *J Biol Chem* **267:** 10127–10132, 1992.
29. Raymond JR, Protein kinase C induces phosphorylation and desensitization of the human 5-HT_{1A} receptor. *J Biol Chem* **266:** 14747–14753, 1991.
30. Hamon M and Gozlan H, Les récepteurs centraux de la sérotonine. *Medecine/Science* **9:** 21–30, 1993.
31. Watson S and Arkinstall, 5-HT receptors. In: *The G-protein linked receptors* (Facts Book Series), pp. 159–181. Academic Press, London, 1994.
32. Gobet I, Durocher Y, Leclerc C, Moreau M and Guerrier P, Reception and transduction of the serotonin signal responsible for meiosis reinitiation in oocytes of the Japanese clam, *Ruditapes philippinarum*. *Dev Biol* **164:** 540–549, 1994.