Biochemical Pharmacology, Vol. 42, No. 4, pp. 899–904, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

REDUCTION OF THE FERTILIZING CAPACITY OF SEA URCHIN SPERM BY CANNABINOIDS DERIVED FROM MARIHUANA

III. ACTIVATION OF PHOSPHOLIPASE A_2 IN SPERM HOMOGENATE BY Δ^9-TETRAHYDROCANNABINOL

MICHAEL C. CHANG,* DIANA BERKERY,* SUZANNE G. LAYCHOCK† and HERBERT SCHUEL*‡

*Department of Anatomical Sciences, and †Department of Pharmacology and Therapeutics, School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, NY 14214, U.S.A.

(*Received* 8 *December* 1990; *accepted* 27 *March* 1991)

Abstract—Inhibition of the egg jelly induced acrosome reaction by Δ^9-tetrahydrocannabinol (THC) is associated with the localized disruption of the nuclear envelope and the formation of lipid deposits in sea urchin sperm. This suggests that THC may activate phospholipase(s) within the sperm. We now report effects of THC on phospholipase A_2 activity in homogenates of sea urchin sperm using 1-stearoyl-2-[1-^{14}C]arachidonyl phosphatidylcholine as substrate. The release of radioactive arachidonic acid was measured after a 30-min incubation with the enzyme. In the absence of exogenous Ca^{2+}, 100 μM THC produced a significant ($P < 0.001$) increase in phospholipase A_2 activity. THC activated phospholipase A_2 in a concentration (1–100 μM) and time-dependent (0–30 min) manner. Exogenous calcium (10 mM) significantly augmented basal ($P < 0.001$) and THC-stimulated ($P < 0.005$) phospholipase A_2 activity. Calcium chelators [ethylene glycol bis(β-aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA) and 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA)] inhibited the basal level of phospholipase A_2 activity in the sperm homogenate, and prevented the activation of phospholipase A_2 by THC. Submicromolar levels of free calcium ions were required for THC stimulation of phospholipase A_2. Cannabinol which mimics the effects of THC on the acrosome reaction also activated phospholipase A_2 in sperm homogenate. These results suggest that THC may alter lipid metabolism in sperm by activating calcium-dependent phospholipase A_2. Putative metabolites derived from this process may inhibit the acrosome reaction and thereby reduce the fertilizing capacity of sea urchin sperm.

Δ^9-Tetrahydrocannabinol (THC) is the major psychoactive cannabinoid extracted from marihuana [1]. THC affects cellular function by reacting with a specific receptor [2–4] and/or by perturbing the structure of membrane lipids [5, 6]. We are using sea urchin gametes as an *in vitro* model system to study the direct effects of THC on fertilization. THC (0.1 to 100 μM) inhibits fertilization in the sea urchin *Strongylocentrotus purpuratus* by reducing the fertilizing capacity of sperm [7]. The motility of THC-treated sperm is not impaired. THC reduces the fertilizing capacity of sperm by blocking the acrosome reaction that normally is stimulated by a specific ligand in the jelly coat of the egg [8]. The acrosome reaction involves exocytosis of the acrosomal granule at the apex of the sperm head. This secretory process exposes the sperm membrane that will attach to and fuse with the egg [9–11]. Ultrastructural studies of THC-treated sperm reveal that blockade of the acrosome reaction is associated with the localized disruption of the nuclear envelope and the formation of lipid deposits within the subacrosomal and centriolar fossae [12]. These morphological observations suggest that THC alters lipid metabolism within the sperm. Cannabinoids activate phospholipase A_2 activity and promote the release of arachidonic acid from membrane phospholipids in a variety of mammalian somatic cells [13–18]. Sea urchin sperm are known to contain phospholipase A_2 activity [19–22]. We now report that the addition of THC to homogenate of sea urchin sperm activates phospholipase A_2 activity assayed using phosphatidylcholine labeled with radioactive arachidonic acid in the second acyl position as substrate.

‡ Correspondence: Dr. Herbert Schuel, Department of Anatomical Sciences, University at Buffalo, SUNY, Buffalo, NY 14214.

METHODS

Sea urchins (*S. purpuratus* and *Lytechinus pictus*) were obtained from Pacific BioMarine Laboratories (Venice, CA). Sperm were collected and assayed for their viability as described previously [7]. Sperm (200 μL) were sonicated (Kontes Cell Disrupter at a setting of 2.5 [3.3 total W] for 20 sec) in 250 μL of ice-cold 0.3 M sucrose in 10 mM HEPES buffer at pH 7.4. The protein concentration of the homogenate was determined using the bicinchoninic acid assay [23].

THC and cannabinol (CBN) were provided by the National Institute of Drug Abuse. The cannabinoids were dissolved in 95% ethanol to a concentration of 200 mg/mL. Propylene glycol was used as the

secondary solvent to prepare working solutions of THC in sucrose–HEPES buffer [7]. Vehicle (solvent) controls contained equivalent amounts of ethanol and propylene glycol in buffered sucrose.

Phospholipase A_2 activity was determined using 1-stearoyl-2-[1-^{14}C]arachidonyl phosphatidylcholine ([^{14}C]PC) as substrate (sp. act. 56 mCi/mmol). The substrate was dried by evaporation under argon, and then dispersed by sonication into 0.5 M Tris at pH 8.0. THC or vehicle was added to the sperm homogenate (100 μg protein) in sucrose–HEPES. The enzyme reaction was started by addition of substrate (50 μL) to the mixture (0.02 μCi radiolabeled phosphatidylcholine, 125 mM Tris buffer, pH 8.0, in a final total volume of 200 μL) at 17°. The reaction was stopped by the addition of 3.0 mL chloroform:methanol (1:2, v/v) for extraction of lipids, and then washed with 1.5 mL distilled water, 1.0 mL chloroform and 100 μL of 1N HCl. The lower phase, containing the extracted lipids was removed, dried under nitrogen gas, redissolved in chloroform:methanol, and applied as a spot onto activated silica gel G coated plates. The separation of free arachidonic acid from the other lipid constituents was carried out by TLC using the solvent system of petroleum ether:diethyl ether:acetic acid (30:20:0.5, by vol.). To separate lysophospholipids from the radioactive substrate, the TLC plates were dried and then re-developed using the solvent system of chloroform:methanol:acetic acid:distilled water (30:25:7.5:0.75, by vol.). Lipid spots were visualized by exposing the dried plate to iodine vapor and/or X-ray film, and identified by comparison with known standards (phosphatidylcholine, arachidonic acid, lysophosphatidylcholine, and diolein for di-glycerides). The lipid fractions were removed from the plate and assayed for radioactivity by scintillation spectrometry [18, 24]. Total recovery of radioactivity was 96.6 ± 3.0%.

Calcium buffers using 1,2-bis(*O*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) as chelating ligand were used to regulate the concentration of free calcium [Ca^{2+}] in the phospholipase assay [25]. Estimates of [Ca^{2+}] in our Ca^{2+}–BAPTA buffers were calculated using the apparent calcium affinity constant (K'_{Ca}) for the ligand according to the quadratic equation:

$$[Ca^{2+}]^2 \cdot K'_{Ca} + [Ca^{2+}] \cdot \{1 + K'_{Ca}([Ligand_T] - [Ca_T])\} - [Ca_T] = 0$$

where $a = K'_{Ca}$, $b = \{1 + K'_{Ca}([Ligand_T] - [Ca_T])\}$ and $c = -[Ca_T]$ with a, b, and c having their usual meaning [25]; T = total. For this calculation we used $K'_{Ca} = 1 \times 10^6\ M^{-1}$. This apparent affinity constant value was determined experimentally at pH 7.0 at 22° at an ionic strength of 0.1 [25]. It is the closest measured K'_{Ca} value to the conditions employed in this study. Any extrapolation below 0.1 M ionic strength probably would cause a larger error (log $K'_{Ca} = 6.97$ from a linear curve to log $K'_{Ca} = 26.07$ from a power curve, coefficient of regression = 0.93 and 0.98, respectively) than using the value actually measured at 0.1 M. Our calculations indicate that K'_{Ca} does not change significantly at 17° compared to 22° (log K'_{Ca} 5.96 vs 6.0, respectively) using equations to compensate for temperature effects [25].

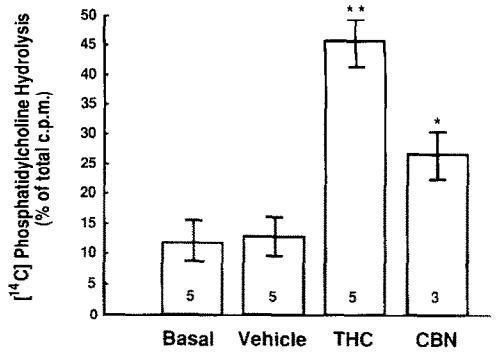

Fig. 1. Stimulation of phospholipase A_2 activity in sea urchin (*S. purpuratus*) sperm homogenate by THC and CBN. [^{14}C]Arachidonic acid release from 1-stearoyl-2-[1-^{14}C]arachidonyl phosphatidylcholine was determined after a 30-min incubation (17°) with homogenate in buffer (basal), vehicle, THC (100 μM) or CBN (100 μM), as indicated. [^{14}C]Arachidonic acid was isolated by TLC. Values are the means ± SEM for the number of determinations shown at the base of each bar. Key: (*) $P < 0.05$, and (**) $P < 0.001$ compared to vehicle-treated control values, as determined by Student's *t*-test.

The lipid standards were obtained from Serdary Research Laboratories (London, Ontario, Canada). Ethylene glycol bis (β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), Tris, and HEPES were obtained from the Sigma Chemical Co. (St. Louis, MO). Radiolabeled phosphatidylcholine was purchased from Amersham (Arlington Heights, IL), BAPTA was obtained from Calbiochem (San Diego, CA), bicinchoninic acid (BCA reagent) was purchased from Pierce (Rockford, IL), and TLC plates were obtained from Whatman (Kent, U.K.).

Data shown are mean values ± SEM for N number of experiments. For each experiment, sperm were pooled from at least three males. The statistical significance of the data was evaluated using Student's *t*-test and tests for analysis of variance (ANOVA) [26].

RESULTS

In previous studies we showed that maximal inhibition of the acrosome reaction is obtained with cannabinoids at a concentration of 100 μM, and the adverse effects of cannabinoids on the acrosome reaction and sperm-fertilizing capacity are reversible [8, 12]. Based upon these findings, we used 100 μM cannabinoid in this study.

Incubation of *S. purpuratus* sperm homogenate with [^{14}C]PC resulted in the release of 12.2 ± 3.2% (N = 5) of [^{14}C]arachidonic acid from the substrate (Fig. 1), indicating the presence of phospholipase A_2 activity. Radioactive hydrolysis product liberated from the substrate was quantitatively recovered in the arachidonic acid fraction (97.3 ± 3.2%), suggesting little or no hydrolysis of [^{14}C]PC by

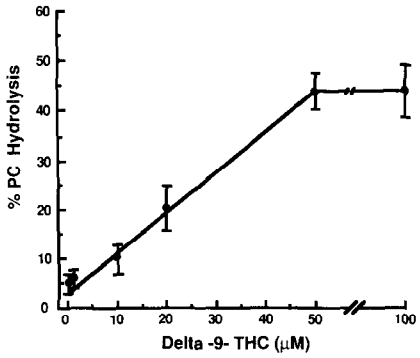

Fig. 2. Activation of phospholipase A_2 activity in sea urchin (*S. purpuratus*) sperm homogenate by THC (1–100 μM). Free arachidonic acid was assayed after a 30-min incubation with sperm homogenate at 17°. Coefficient of regression between 1 and 50 μM THC: 0.99. Rates of hydrolysis for 10–100 μM THC were statistically significant ($P < 0.05$ to $P < 0.002$ according to Student's *t*-test) compared to the basal rate in the absence of THC. Values are the means ± SEM for 4 experiments.

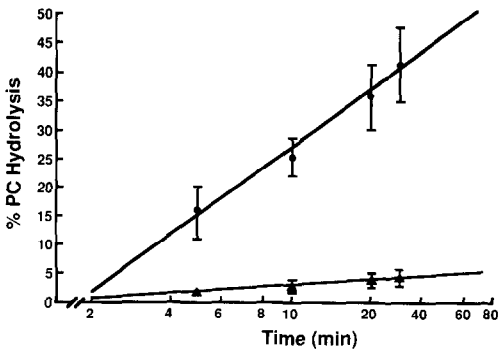

Fig. 3. Time dependence of phospholipase A_2 activity following addition of THC (100 μM) to sperm (*S. purpuratus*) homogenate. Key: THC (●), and vehicle (▲). Coefficient of regression for THC: 1.0. Curves for THC and vehicle were significantly different ($P < 0.025$) based on ANOVA. Values are the means ± SEM for 4 experiments.

hydrolytic enzymes other than phospholipase A_2. Less than 1.0% of the radioactivity was recovered in the lysophosphatidylcholine and diglyceride fractions. Substrate hydrolysis in the absence of homogenate was less than 0.3%.

The addition of THC to the sperm homogenate stimulated phospholipase A_2 activity, as evidenced by a significant increase in [^{14}C]arachidonic acid levels (Fig. 1), but no change in [^{14}C]diglyceride or [^{14}C]lysophosphatidylcholine levels (data not shown). Phospholipase A_2 activity was increased 3-fold with 100 μM THC, whereas the vehicle did not affect enzyme activity. The effect of THC on phospholipase A_2 was concentration and time dependent (Figs. 2 and 3). The hydrolysis of [^{14}C]PC increased in a linear manner with increasing THC concentrations (1–50 μM) (Fig. 2). THC-treated sperm homogenate [^{14}C]PC hydrolysis was consistently elevated throughout the 30-min incubation (Fig. 3). Another cannabinoid, CBN, which mimics the effects of THC on sperm-fertilizing capacity, the acrosome reaction, and sperm ultrastructure [7, 8, 12], also stimulated phospholipase A_2 activity in sperm homogenate (Fig. 1).

Phospholipase A_2 in somatic cells is a Ca^{2+}-dependent enzyme [27]. In the experiments described above, the maximum total calcium carried over into the assay with the homogenate was 8.2 ± 0.5 μM based upon the calcium concentration in seminal plasma [28]. When exogenous Ca^{2+} (10 mM) was added to the vehicle-treated homogenate, phospholipase A_2 activity was increased by more than 5-fold ($P < 0.001$) (Table 1). The addition of exogenous Ca^{2+} also significantly augmented phospholipase A_2 activity in the presence of THC ($P < 0.005$). In the presence of 10 mM Ca^{2+}, [^{14}C]PC hydrolysis was not different for control sperm homogenate and THC-supplemented homogenate.

The role of calcium in supporting phospholipase A_2 activity also was studied using calcium chelators. EGTA is a chelator with a high specificity for calcium compared to other divalent cations [29]. Addition of EGTA to chelate endogenous calcium resulted in the complete inhibition of the basal and the THC-stimulated phospholipase A_2 activity in homogenate of *S. purpuratus* sperm (Table 1). Calcium buffers were employed to further define the calcium requirement for the THC-stimulated phospholipase A_2 in sperm. We used BAPTA for these experiments because its affinity for calcium is not sensitive to changes in pH between 7 and 8, whereas EGTA exhibits an appreciable pH sensitivity over this range [25]. Results obtained with Ca^{2+}-BAPTA buffers show that submicromolar levels of free calcium ions were required for THC stimulation of phospholipase A_2 in homogenate of *L. pictus* sperm (Fig. 4). Addition of BAPTA (1 mM) to chelate endogenous calcium completely inhibited the basal and the THC-stimulated phospholipase A_2 in *L. pictus* sperm homogenate (Table 2). In the absence of BAPTA, maximal THC-stimulated phospholipase A_2 activity was obtained with 1 and 10 mM exogenous calcium. Taken together these findings show that the THC-stimulated phospholipase A_2 in sea urchin sperm is a calcium-dependent enzyme.

DISCUSSION

Our findings show that THC and CBN activated phospholipase A_2 in homogenate of sea urchin sperm. The TLC assay that we employed was designed to detect radioactive hydrolysis products released by the actions of phospholipase A_2 on phosphatidylcholine with [^{14}C]arachidonic acid in the second acyl position ([^{14}C]PC). Over 97% of the radioactivity released from the substrate was recovered in the arachidonic acid fractions. This finding is consistent with the expected action of phospholipase A_2. Arachidonic acid also can be released from the second acyl position of phospholipids by the sequential actions of phos-

Table 1. Effect of EGTA on stimulation of phospholipase A_2 activity in sea urchin (*S. purpuratus*) sperm homogenate by 100 μM THC

Added calcium (10 mM)	EGTA (1 mM)	Phosphatidylcholine hydrolysis (%) THC	Vehicle	% Increase (THC/vehicle)
–	–	41.4 ± 4.2	10.0 ± 2.0	406.9 ± 88.3
–	+	0.2 ± 0.02 ($P < 0.001$)	0.2 ± 0.02 ($P < 0.01$)	50.0 ± 22.4
+	–	68.0 ± 5.4	58.9 ± 7.7	37.2 ± 26.3
+	+	64.6 ± 9.1 ($P > 0.7$)	46.7 ± 9.7 ($P > 0.3$)	56.8 ± 27.6

P values in parentheses were calculated by Student's *t*-test comparing PC hydrolysis for −EGTA (N = 10) vs +EGTA (N = 5) in THC and vehicle. Values are means ± SEM for the indicated number of experiments.

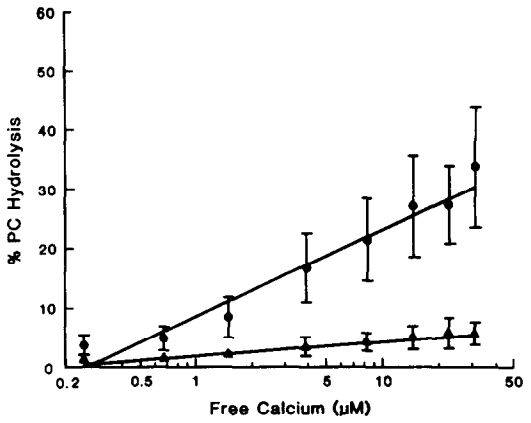

Fig. 4. Effect of free calcium on THC (100 μM) stimulated phospholipase A_2 activity in sea urchin (*L. pictus*) sperm homogenate using Ca^{2+}-BAPTA buffers. Free calcium concentration was calculated as described in the Methods. The buffer solutions contained 1 mM BAPTA plus 0.2, 0.4, 0.6, 0.8, 0.9, 0.95, 0.98 or 1.0 mM added calcium. Key: THC (●), and vehicle (▲). Coefficient of regression for THC was 0.96 and 0.97 for vehicle. Curves for THC and vehicle were significantly different ($P < 0.005$) based on ANOVA. Values are the means ± SEM for 5 experiments.

pholipase A_1 and lysophospholipase, or by phospholipase C and diacylglycerol lipase [30]. The absence of significant radioactivity within the lysophosphatidylcholine fractions indicates that the phospholipase A_1 pathway is not operative. This observation is consistent with previous studies on phospholipase A_2 in sperm of other species of sea urchins [21, 22] as well as in human sperm [30]. The absence of radioactivity within the diglyceride fractions shows that phospholipase C, which is present in sea urchin sperm [31], does not hydrolyze [^{14}C]PC. We have not ruled out the possibility that under appropriate conditions using a preferred substrate such as phosphosphatidylinositol that THC also might affect phospholipase C activity. The THC-stimulated phospholipase A_2 activity was augmented by addition of exogenous calcium, and suppressed by addition of calcium chelators: EGTA and BAPTA. Submicromolar levels of free calcium ions were required for THC activation of the enzyme. THC may affect the free calcium levels in the system, or alter the availability of calcium necessary for the enzyme–substrate reaction. An increase in phospholipase A_2 activity may account for the localized disruption of the nuclear envelope and accumulation of lipid deposits in sea urchin sperm associated with the blockade of the acrosome reaction by cannabinoids [8, 12].

Table 2. Effect of BAPTA on stimulation of phospholipase A_2 activity in sea urchin (*L. pictus*) sperm homogenate by 100 μM THC

Added calcium (mM)	BAPTA (1 mM)	N	Phosphatidylcholine hydrolysis (%) THC	Vehicle	% Increase (THC/Vehicle)
0.0	–	8	53.5 ± 4.6	21.6 ± 4.7	258.3 ± 86.2
0.0	+	8	1.7 ± 0.7 ($P < 0.001$)	0.9 ± 1.1 ($P < 0.001$)	133.8 ± 37.0
1.0	–	6	64.4 ± 1.8	42.5 ± 7.2	96.8 ± 55.6
1.0	+	4	34.0 ± 10.3 ($P < 0.01$)	5.6 ± 3.8 ($P > 0.005$)	594.6 ± 241.1
10.0	–	8	64.6 ± 1.8 [$P > 0.7$]	43.7 ± 5.3 [$P > 0.8$]	65.4 ± 20.6

P values in parentheses were calculated by Student's *t*-test comparing PC hydrolysis for −BAPTA vs +BAPTA in THC and vehicle. P values in brackets were calculated comparing PC hydrolysis for 1.0 mM vs 10.0 mM added calcium in THC and vehicle. Values are means ± SEM for the indicated number of experiments.

Arachidonic acid released from cellular membranes by phospholipase A_2 can be oxidized enzymatically by cells to generate highly potent membrane permeable bioregulatory metabolites (prostaglandins, thromboxanes, leukotrienes, hydroxyeicosatetraenoic acids, epoxides) through the arachidonic acid cascade [27, 32, 33]. THC and other cannabinoids activate the arachidonic acid cascade in mammalian somatic cells, and this may be a common cellular mechanism for promoting the diverse biological effects of cannabinoids [13–17, 34–38]. Based upon our observations and evidence in the literature, we postulate that enhanced generation of arachidonic acid metabolites caused by THC may be responsible in part for inhibition of the acrosome reaction which reduces the capacity of sperm to fertilize eggs. This hypothesis is consistent with previous studies implicating the arachidonic acid cascade in modulating gamete interactions during fertilization [39–41]. Future studies are required to identify arachidonic acid derived metabolites that may be produced in sea urchin sperm.

The acrosome reaction in sea urchin [9–11] and mammalian [42] sperm is a ligand-stimulated exocytotic event. It is possible that THC and other cannabinoids may affect this process during fertilization in humans. It is interesting to note that the receptors which trigger the acrosome reaction in mammalian and invertebrate sperm [43], the activation of phospholipase A_2 in somatic cells [27, 44, 45], and cannabinoids in the brain [2] are G-protein-coupled. Receptors may also mediate the effects of THC on sperm phospholipase A_2 [this study], ultrastructure [12], acrosome reaction [8], and fertilizing capacity [7, 8]. Alternatively, THC may activate phospholipase A_2 by altering the fluidity of membrane lipids [5, 6], and the accessibility of the enzyme to its substrate and/or cation.

THC blocks the ligand (egg jelly)-stimulated acrosome reaction in sea urchin sperm. However, it does not inhibit the acrosome reaction induced by ionophores such as ionomycin [8]. This suggests that THC affects an event(s) in the stimulation–secretion–coupling mechanism of the sperm. THC similarly inhibits secretion in the exocrine pancreas by affecting signal transduction [46]. In both cases the THC-stimulated increase in free arachidonic acid may be related to the formation of a metabolite(s) that acts as a second messenger to block signal transduction. In the future it would be useful to extend these studies to intact sperm cells, isolated organelles, and membrane fractions to further define the physiological role of the THC-stimulated phospholipase A_2 in modulating the acrosome reaction. Elucidation of the mechanisms responsible for the THC-induced blockade of the acrosome reaction in sea urchins may contribute to our understanding of how cannabinoids affect cellular regulation in other systems.

Acknowledgements—This research was supported by USPHS grants from the National Institute of Drug Abuse (DA-05000) to H.S. and the National Institute of Diabetes, Digestive and Kidney Diseases (DK-25705) to S.G.L. We thank Dr. J. Klingman for his assistance with the TLC, and Dr. A. Fabiato for his assistance with the estimation of free calcium in the Ca^{2+}-BAPTA buffers.

REFERENCES

1. Mechoulam R, Srebnik N and Burstein S, Cannabis chemistry, biochemistry and therapeutic application—an overview. In: *Marihuana '84: Proceedings of the Symposium on Cannabis* (Ed. Harvey DJ), pp. 1–12. IRL Press, Oxford, 1985.
2. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC and Bonner TI, Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**: 561–564, 1990.
3. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, deCosta BR and Rice KC, Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* **87**: 1932–1936, 1990.
4. Howlett AC, Johnson MR, Melvin LS and Milne GM, Nonclassical cannabinoid analgetics inhibit adenylate cyclase: Development of a cannabinoid receptor model. *Mol Pharmacol* **33**: 297–302, 1988.
5. Hillard CJ, Harris RA and Bloom AS, Effects of the cannabinoids on physical properties of brain membranes and phospholipid vesicles: Fluorescence studies. *J Pharmacol Exp Ther* **232**: 579–588, 1985.
6. Lawrence DK and Gill EW, The effects of Δ^1-tetrahydrocannabinol and other cannabinoids on spin-labeled liposomes and their relationships to mechanisms of general anesthesia. *Mol Pharmacol* **11**: 595–602, 1975.
7. Schuel H, Schuel R, Zimmerman AM and Zimmerman S, Cannabinoids reduce fertility of sea urchin sperm. *Biochem Cell Biol* **65**: 130–136, 1987.
8. Schuel H, Berkery D, Schuel R, Chang MC, Zimmerman AM and Zimmerman S, Reduction of the fertilizing capacity of sea urchin sperm by cannabinoids derived from marihuana. I. Inhibition of the acrosome reaction induced by egg jelly. *Mol Reprod Dev* **29**: 51–59, 1991.
9. Dan J, Ohori Y and Kushida H, Studies of the acrosome. VII. Formation of the acrosomal process in sea urchin spermatozoa. *J Ultrastruct Res* **11**: 508–524, 1964.
10. SeGall GK and Lennarz WJ, Chemical characterization of the component of the jelly coat from sea urchin eggs responsible for induction of the acrosome reaction. *Dev Biol* **71**: 33–48, 1979.
11. Kopf GS and Garbers DL, Calcium and a fucose-sulfate-rich polymer regulate sperm cyclic nucleotide metabolism and the acrosome reaction. *Biol Reprod* **22**: 1118–1126, 1980.
12. Chang MC and Schuel H, Reduction of the fertilizing capacity of sea urchin sperm by cannabinoids derived from marihuana. II. Ultrastructural changes associated with inhibition of the acrosome reaction. *Mol Reprod Dev* **29**: 60–71, 1991.
13. Burstein S and Hunter SA, Prostaglandins and cannabis—VI. Release of arachidonic acid from HeLa cells by Δ^1-tetrahydrocannabinol and other cannabinoids. *Biochem Pharmacol* **27**: 1275–1280, 1978.
14. Burstein S and Hunter SA, Prostaglandins and cannabis—VII. Elevation of phospholipase A_2 activity by cannabinoids in whole cells and subcellular preparations. *J Clin Pharmacol* **21**: 240S–248S, 1981.
15. Burstein S, Hunter SA, Ozman K and Renzulli L, Prostaglandins and cannabis—XIII. Cannabinoid-induced elevation of lipoxygenase products in mouse peritoneal macrophages. *Biochem Pharmacol* **33**: 2653–2656, 1984.
16. Hunter SA, Burstein S and Sedor C, Stimulation of prostaglandin synthesis in WI-38 human lung fibroblasts following inhibition of phospholipid acylation by *p*-hydroxymercuribenzoate. *Biochim Biophys Acta* **793**: 202–212, 1984.

17. Hunter SA, Burstein S and Renzulli L, Effects of cannabinoids on the activities of mouse brain lipases. *Neurochem Res* **11**: 1273–1288, 1986.
18. Laychock SG, Hoffman JM, Meisel E and Bilgin S, Pancreatic islet arachidonic acid turnover and metabolism and insulin release in response to delta-9-tetrahydrocannabinol. *Biochem Pharmacol* **35**: 2003–2008, 1986.
19. Conway AF and Metz CB, Phospholipase activity of sea urchin sperm: Its possible involvement in membrane fusion. *J Exp Zool* **198**: 39–48, 1976.
20. SeGall GK and Lennarz WJ, Jelly coat and induction of the acrosome reaction in echinoid sperm. *Dev Biol* **86**: 87–93, 1981.
21. Mita M and Ueta N, Energy metabolism of sea urchin spermatozoa with phosphatidylcholine as the preferred substrate. *Biochim Biophys Acta* **959**: 361–369, 1988.
22. Mita M, Energy metabolism of spermatozoa of the sea urchin, *Glyptocidaris crenularis*. *Mol Reprod Dev* **28**: 280–285, 1991.
23. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**: 76–85, 1985.
24. Laychock SG, Franson RC, Weglicki WB and Rubin RP, Identification and partial characterization of phospholipases in isolated adrenocortical cells. *Biochem J* **164**: 753–756, 1977.
25. Harrison SM and Bers DM, The effect of temperature and ionic strength on the apparent Ca-affinity of EGTA and the analogous Ca-chelators BAPTA and dibromo-BAPTA. *Biochim Biophys Acta* **925**: 133–143, 1987.
26. Snedecor GW and Cochran WG, *Statistical Methods*, 7th Edn. Iowa State University Press, Ames, IA, 1979.
27. Axelrod J, Burch RM and Jelsema CL, Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins: Arachidonic acid and its metabolites as second messengers. *Trends Neurosci* **11**: 117–123, 1988.
28. Vacquier VD, Handling, labeling, and fractionating sea urchin spermatozoa. *Methods Cell Biol* **27**: 15–40, 1986.
29. Kim YS and Padilla GM, Determination of free Ca ion concentrations with an ion-selective electrode in the presence of chelating agents in comparison with calculated values. *Anal Biochem* **89**: 521–528, 1978.
30. Anderson RA Jr, Johnson SK, Bielfeld P, Feathergill KA and Zaneveld LJD, Characterization and inhibitor sensitivity of human sperm phospholipase A_2: Evidence against pivotal involvement of phospholipase A_2 in the acrosome reaction. *Mol Reprod Dev* **27**: 305–325, 1990.
31. Domino SE and Garbers DL, The fucose-sulfate glycoconjugate that induces an acrosome reaction in spermatozoa stimulates inositol 1,4,5-trisphosphate accumulation. *J Biol Chem* **263**: 690–695, 1988.
32. Laychock SG and Putney JW, Roles of phospholipid metabolism in secretory cells. In: *Cellular Regulation of Secretion and Release* (Ed. Conn PM), pp. 53–105. Academic Press, New York, 1982.
33. Needleman P, Turk J, Jakschik BA, Morrison AR and Lefkowith JB, Arachidonic acid metabolism. *Annu Rev Biochem* **55**: 69–102, 1986.
34. Burstein S, Ozman K, Burstein E, Palermo N and Smith E, Prostaglandins and cannabis—XI. Inhibition of Δ^1-tetrahydrocannabinol-induced hypotension by aspirin. *Biochem Pharmacol* **31**: 591–592, 1982.
35. Burstein S, Hunter SA, Sedor C and Shulman S, Prostaglandins and cannabis—IX. Stimulation of prostaglandin E_2 synthesis in human lung fibroblasts by Δ^1-tetrahydrocannabinol. *Biochem Pharmacol* **31**: 2361–2365, 1982.
36. Burstein S, Hunter SA and Renzulli L, Prostaglandins and cannabis XIV. Tolerance to the stimulatory actions of cannabinoids on arachidonate metabolism. *J Pharmacol Exp Ther* **235**: 87–91, 1985.
37. Burstein SH, Hull K, Hunter SA and Shilstone J, Immunization against prostaglandins reduces Δ^1-tetrahydrocannabinol-induced catalepsy in mice. *Mol Pharmacol* **35**: 6–9, 1989.
38. White HL and Tansik RL, Effects of Δ^9-tetrahydrocannabinol and cannabidiol on phospholipase and other enzymes regulating arachidonate metabolism. *Prostaglandins Med* **4**: 409–411, 1980.
39. Schuel H, Moss R and Schuel R, Induction of polyspermic fertilization in sea urchins by the leukotriene antagonist FPL-55712 and the 5-lipoxygenase inhibitor BW755C. *Gamete Res* **11**: 41–50, 1985.
40. Schuel H, Traeger E, Schuel R and Boldt J, Anti-inflammatory drugs promote polyspermic fertilization in sea urchins. *Gamete Res* **10**: 9–19, 1984.
41. Schuel H, The prevention of polyspermic fertilization in sea urchins. *Biol Bull* **167**: 271–309, 1984.
42. Leyton L and Saling P, Evidence that aggregation of mouse sperm receptors by ZP3 triggers the acrosome reaction. *J Cell Biol* **108**: 2163–2168, 1989.
43. Kopf GS, Woolkalis MJ and Gerton GL, Evidence for a guanine nucleotide-binding regulatory protein in invertebrate and mammalian sperm. *J Biol Chem* **261**: 7327–7331, 1986.
44. Kim D, Lewis DL, Graziadei L, Neer EJ, Bar-Sagi D and Clapham DE, G-protein $\beta\gamma$-subunits activate the cardiac muscarinic K^+-channel via phospholipase A_2. *Nature* **337**: 557–560, 1989.
45. Gupta SK, Diez E, Heasley LE, Osawa S and Johnson GL, A G-protein mutant that inhibits thrombin and purinergic receptor activation of phospholipase A_2. *Science* **249**: 662–666, 1990.
46. Chaudhry A, Thompson RH, Rubin RP and Laychock SG, Relationship between Δ-9-tetrahydrocannibinol-induced arachidonic acid release and secretagogue-evoked phosphoinositide breakdown and Ca^{2+} mobilization of exocrine pancreas. *Mol Pharmacol* **34**: 543–548, 1988.