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Anandamide (arachidonylethanolamide), a brain cannabinoid receptor agonist, reduces sperm fertilizing capacity in sea urchins by inhibiting the acrosome reaction

(fertilization/secretion/arachidonic acid/marihuana/Δ^9-tetrahydrocannabinol)

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ABSTRACT **Anandamide (arachidonylethanolamide) is an endogenous cannabinoid receptor agonist in mammalian brain. Sea urchin sperm contain a high-affinity cannabinoid receptor similar to the cannabinoid receptor in mammalian brain. (−)-Δ^9-Tetrahydrocannabinol (THC), the primary psychoactive cannabinoid in marihuana, reduces the fertilizing capacity of sea urchin sperm by blocking the acrosome reaction that normally is stimulated by a specific ligand in the egg's jelly coat. We now report that anandamide produces effects similar to those previously obtained with THC in *Strongylocentrotus purpuratus* in reducing sperm fertilizing capacity and inhibiting the egg jelly-stimulated acrosome reaction. Arachidonic acid does not inhibit the acrosome reaction under similar conditions. The adverse effects of anandamide on sperm fertilizing capacity and the acrosome reaction are reversible. The receptivity of unfertilized eggs to sperm and sperm motility are not impaired by anandamide. Under conditions where anandamide completely blocks the egg jelly-stimulated acrosome reaction, it does not inhibit the acrosome reaction artificially initiated by ionomycin, which promotes Ca^{2+} influx, and nigericin, which activates K^+ channels in sperm. These findings provide additional evidence that the cannabinoid receptor in sperm plays a role in blocking the acrosome reaction, indicate that anandamide or a related molecule may be the natural ligand for the cannabinoid receptor in sea urchin sperm, and suggest that binding of anandamide to the cannabinoid receptor modulates stimulus–secretion-coupling in sperm by affecting an event prior to ion channel opening.**

Sea urchin sperm contain a cannabinoid receptor that is similar to the cannabinoid receptor in mammalian brain (1). The primary psychoactive compound in marihuana is (−)-Δ^9-tetrahydrocannabinol (THC) (2). THC directly inhibits fertilization in the sea urchin by reducing the fertilizing capacity of sperm (3, 4). The receptivity of the unfertilized egg to sperm and the motility of sperm are not impaired by the drug. THC reduces the fertilizing capacity of the sperm by blocking the acrosome reaction that normally is stimulated by a specific ligand in the jelly coat of the egg (4, 5). When THC is removed from pretreated sperm their capacity to undergo the acrosome reaction is restored along with their ability to fertilize eggs. Under conditions where THC completely blocks the egg jelly-stimulated acrosome reaction, it does not inhibit the acrosome reaction artificially initiated by ionophores that promote specific fluxes of calcium and potassium ions. Our findings suggest that the cannabinoid receptor in sea urchin sperm is involved in blockade of the acrosome reaction by THC and other cannabinoids (1).

Sea urchin sperm represent a unique model system to study the stimulation–secretion-coupling mechanism. The acrosome is a specialized secretory granule at the apex of the sperm head. In sea urchins the acrosome reaction is triggered by the binding of an egg jelly-derived ligand with a species-specific receptor in the plasma membrane of the sperm head to trigger exocytosis of the acrosomal granule (6–8, 59). This secretory process is essential for fertilization in sea urchins and mammals because it exposes the sperm membrane that will attach to and fuse with the egg (6, 9). Early responses detected in sperm after the egg jelly ligand binds to its receptor in the plasma membrane of the sperm head are identical to those associated with signal transduction mechanisms in mammalian cells after stimulation by neurotransmitters, hormones, growth factors, etc. (8). THC and other cannabinoids block the egg jelly-stimulated exocytosis of the acrosomal granule in sea urchin sperm (5). Since sea urchin sperm contain a cannabinoid receptor (1), these features may make it possible to determine how the receptor directly affects the process of secretion.

Anandamide (arachidonylethanolamide) has been identified as a natural endogenous ligand for the cannabinoid receptor in brain (10). It may serve as a genuine neurotransmitter for cannabinoid receptors (11, 12). Injection of anandamide and THC into mice produces similar biological effects (13). Pharmacologically active fatty acid ethanolamides have been found in plants, egg yolk, and several mammalian tissues (14–18). The presence of a functional cannabinoid receptor in sea urchin sperm implies the presence of an endogenous ligand (1). We now report that anandamide produces effects similar to those previously obtained with THC on sperm fertilizing capacity and the acrosome reaction in the sea urchin.

MATERIALS AND METHODS

Sea urchins, *Strongylocentrotus purpuratus*, were obtained from Pacific Bio-Marine Lab (Venice, CA). The effects of anandamide and THC on gamete fertility and the induction of the acrosome reaction by solubilized egg jelly were assayed as described (1, 3). All experiments were conducted at 15°C in artificial sea water buffered with 5 mM Hepes at pH 7.8 (SW) (1).

THC was supplied in 95% EtOH at a concentration of 200 mg/ml. Anandamide and arachidonic acid (100 mM) in EtOH were stored under nitrogen at −20°C. Thin-layer chromatog-

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Abbreviations: THC, (−)-Δ^9-tetrahydrocannabinol; SW, artificial sea water buffered with 5 mM Hepes at pH 7.8.

raphy (TLC) on silica gel glass plates (0.25-mm-thick) was performed using a solvent system of petroleum ether (bp 38.1–53.7°C)/ether/methanol (6:40:4) to periodically evaluate the purity and stability of the anandamide and arachidonic acid stock solutions (R_f: 0.38 ± 0.02 and 0.74 ± 0.02, respectively; n = 4). Each compound produced only a single spot on TLC, and there was no change in biological potency during storage. Anandamide and arachidonic acid were dissolved directly into SW. Propylene glycol was used as a secondary solvent to prepare working solutions of THC in SW (3). Vehicle controls contained equivalent amounts of the organic solvents in SW. For each experimental series, the concentration of vehicle was maintained at a constant level that was equal to the amount of organic solvents carried over with the highest concentration of drug used. Consistent with previous findings (1, 3–5), the vehicles did not affect gamete functions compared to cells cultured in SW (data not shown). All experimental procedures were conducted in trimethylsilyl (Regisil; Regis, Morton Grove, IL)-treated glassware to prevent the absorption of cannabinoids to the glass surface (19).

Each experiment was repeated three to six times using gametes obtained from different animals. A minimum of 100 cells was counted at random from each culture. Data are presented as mean values ± SEM for n number of trials. Dose–response curves were analyzed by curvilinear regression to estimate 50% inhibition (IC_{50}) values (20). The statistical significance of the data was evaluated using Student's t test and tests for analysis of variance (ANOVA) (21).

THC was graciously provided by the National Institute of Drug Abuse (Bethesda, MD). Anandamide was synthesized in the laboratory of R.M. (10). Arachidonic acid, ionomycin, and nigericin were purchased from Sigma.

RESULTS

Effects on Fertilization. The effects of anandamide on gamete fertility were evaluated (Fig. 1). A minimum sperm density (3.3 ± 0.3 × 10^6 cells per ml, final) just sufficient to fertilize 90–100% after 5 min of preincubation in SW was used. Anandamide did not reduce receptivity of pretreated eggs to sperm. However, pretreatment of sperm with anandamide produced a concentration-dependent reduction in sperm fertilizing capacity. These results are similar to those previously obtained with THC, which inhibits fertilization in sea urchins by reducing the fertilizing capacity of sperm (3). In the present study we also determined the effect of THC on sperm fertility to assess the relative potency of anandamide and THC in reducing sperm fertilizing capacity, IC_{50}: anandamide, 1.23 ± 0.60 μM; THC, 0.41 ± 0.14 μM ($P > 0.2$). The dose–response curves for THC and anandamide also were evaluated by ANOVA ($P > 0.05$). We conclude that the observed differences in potency of anandamide and THC in reducing sperm fertilizing capacity are not statistically significant. Consistent with previous studies with THC (3), the adverse effect of 5 μM anandamide on sperm fertilizing capacity was completely reversible (data not shown).

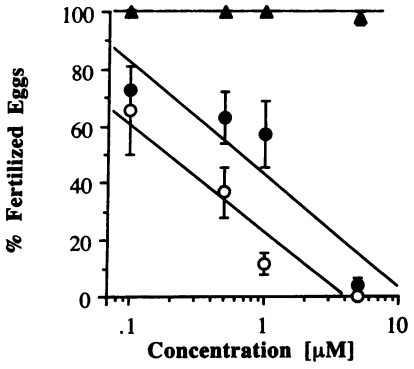

FIG. 1. Effects of anandamide and THC (0.1–5.0 μM) on gamete fertility: receptivity of anandamide-pretreated eggs to sperm (▲) and fertilizing capacity of sperm pretreated with anandamide (●) or THC (○). The receptivity of eggs to sperm was determined by pretreating 0.1 ml of settled eggs in 5 ml of anandamide for 5 min. The cultures were inseminated by addition of 0.1 ml of untreated sperm. The fertilizing capacity of sperm was determined by pretreating 0.1 ml of sperm in 5 ml of anandamide or THC for 5 min prior to addition of 0.1 ml of untreated eggs to the cultures. The cultures were fixed 5 min after insemination. The incidence of fertilized eggs was determined by scoring eggs with elevated fertilization envelopes. Final sperm density, 3.3 ± 0.3 × 10^6 cells per ml. Coefficient of regression for reduction of sperm fertilizing capacity by anandamide, 0.83, and by THC, 0.94. Data on the effects of anandamide on egg fertility and sperm fertilizing capacity were obtained with gametes from the same batches of males and females (n = 6). Reduction of sperm fertilizing capacity by THC (n = 5).

Effects on Acrosome Reaction. The effects of anandamide, THC, and arachidonic acid on triggering of the acrosome reaction were investigated (Fig. 2). Arachidonic acid is a likely precursor of anandamide (17). Pretreatment of sperm (3 × 10^8 cells per ml) with anandamide and THC (0.1–25 μM) produced a concentration-dependent inhibition of the egg jelly-stimulated acrosome reaction, IC_{50}: 8.66 ± 2.12 μM and 4.62 ± 0.19 μM, respectively ($P > 0.1$). The dose–response curves for blockade of the acrosome reaction by anandamide and THC also were evaluated by ANOVA ($P > 0.05$). The observed differences in potency of anandamide and THC are not statistically significant. Arachidonic acid did not inhibit the acrosome reaction under these conditions. Exogenous arachidonic acid reduces the fertility of sea urchin eggs (reviewed in ref. 22). Anandamide did not affect fertility of eggs (see Fig. 1). Thus anandamide and arachidonic acid produce different effects on eggs and sperm. A concentration of 25 μM anandamide is required to block the acrosome reaction in >90% of pretreated sperm cells. Sperm in 25 μM anandamide and THC were observed to swim as actively as control sperm cultured in vehicle or SW for >10 min. Since sea urchin eggs are fertilized within seconds after insemination (22), we conclude that anandamide and THC reduce sperm fertilizing capacity by blocking the acrosome reaction.

The reversibility of the adverse effects of anandamide on the egg jelly-stimulated acrosome reaction (Table 1) and sperm fertilizing capacity (Table 2) were evaluated. In these

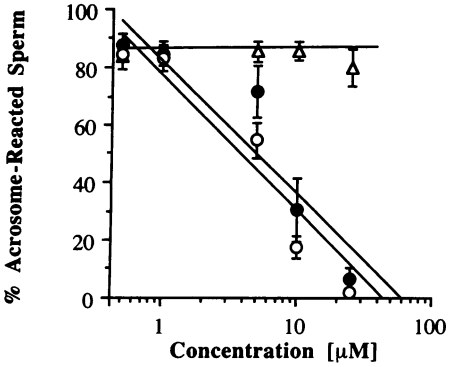

FIG. 2. Effects of anandamide (●), THC (○), and arachidonic acid (△) on stimulation of the acrosome reaction in sperm by egg jelly. Sperm (3 × 10^8 cells per ml) were pretreated for 5 min in 0–25 μM of each compound. Solubilized egg jelly was then added to the cultures (1.2 μg of fucose equivalent per ml, final). The cultures were fixed 3 min later for examination as whole mounts by transmission electron microscopy. Acrosome-reacted sperm were scored on the basis of the presence of the acrosomal process at the apex of the sperm head. Coefficient of regression for blockade of acrosome reaction by anandamide, 0.87, and by THC, 0.94. n = 5.

Table 1. Reversibility of the adverse effect of anandamide on sperm: Egg jelly-induced acrosome reaction

Initial sperm pretreatment conc., μM	SW washout	Acrosome reaction cultures: Final conc., μM	Acrosome reaction cultures: Reacted sperm, %
Anandamide			
25.0	−	25.0	5.0 ± 1.7
25.0	+	0.25	76.5 ± 3.6*
Vehicle equivalent			
25.0	−	25.0	79.3 ± 3.4
25.0	+	0.25	74.3 ± 4.7

Serm (3×10^{10} cells per ml) were pretreated for 5 min in 1.0 ml of anandamide or vehicle in SW. Aliquots (10 μl) of pretreated sperm were transferred to 1.0-ml cultures (3×10^8 cells per ml, final) containing anandamide, vehicle, or SW. After incubation for 5 min, egg jelly (24 μl) was added to the cultures (1.2 μg of fucose per ml, final). The cultures were fixed 3 min later and scored to determine the incidence of acrosome-reacted sperm. $n = 4$.
*$P < 0.01$ determined by Student's t test comparing − vs. + washout of anandamide from pretreated sperm.

experiments pretreatment of sperm with 25 μM anandamide for 5 min inhibited the acrosome reaction by ≈94% (Table 1) and reduced sperm fertilizing capacity by 98% (Table 2). Pretreatment of sperm with equivalent amounts of vehicle did not affect their ability to undergo the acrosome reaction or to fertilize eggs. After the anandamide concentration was reduced 100× by dilution with SW, the sperm regained their ability to undergo the acrosome reaction (Table 1) and to fertilize eggs (Table 2). The recoveries of biological functions after removal of anandamide from pretreated sperm were statistically significant ($P < 0.01$). Similar findings were previously obtained with THC (4).

Ionophores bypass the receptor–ligand reaction with egg jelly and trigger the acrosome reaction by directly opening ion channels in the sperm. Ionomycin promotes influx of Ca^{2+} to initiate the acrosome reaction (23). Nigericin stimulates the acrosome reaction by activating K^+ channels (24). Sperm were pretreated with 25 μM anandamide for 5 min to block the egg jelly-stimulated acrosome reaction by >95% (Table 3). However, this anandamide pretreatment did not suppress the acrosome reaction artificially elicited by ionomycin and nigericin. These findings are similar to those previously obtained with THC (4). Consistent with previous results (4), the organic solvents used to dissolve ionomycin

Table 2. Reversibility of the adverse effect of anandamide on sperm: Fertilizing capacity

Initial sperm pretreatment conc., μM	SW washout	Fertilization cultures: Final conc., μM	Fertilization cultures: Fertilized eggs, %
Anandamide			
25.0	−	25.0	0.6 ± 0.6
25.0	+	0.25	42.0 ± 9.1*
0.25	−	0.25	99.2 ± 0.6
Vehicle equivalent			
25.0	−	25.0	98.6 ± 0.4
25.0	+	0.25	98.0 ± 0.5
0.25	−	0.25	99.2 ± 0.4

Sperm (3×10^8 cells per ml) were pretreated for 5 min in anandamide or vehicle dissolved in SW. Aliquots (50 μl) of the pretreated sperm suspensions were transferred to cultures (5 ml) containing anandamide, vehicle, or SW. Eggs (100 μl) were added to the cultures immediately thereafter (final sperm density: 3×10^6 cells per ml). The cultures were fixed 10 min later and scored to determine the incidence of fertilized eggs. $n = 5$.
*$P < 0.01$ determined by Student's t test comparing fertilizing capacity of sperm − vs. + washout of anandamide from pretreated sperm.

Table 3. Effect of pretreatment of sperm with anandamide on stimulation of acrosome reaction by egg jelly and ionophores

Inducer	Conc.	% acrosome-reacted sperm: Anandamide	% acrosome-reacted sperm: Vehicle
Egg jelly	1.2 μg/ml*	1.5 ± 0.7	94.0 ± 1.4
Ionomycin	10 μM	98.3 ± 0.8	99.8 ± 0.3
Nigericin	50 μM	96.8 ± 1.3	81.5 ± 5.0

Sperm (3×10^8 cells per ml) were pretreated with 25 μM anandamide or vehicle equivalent in SW for 5 min. Egg jelly (24 μl) or ionophore (10 μl) was then added to the cultures. The cultures were fixed 5 min later. $n = 4$.
*1.2 μg of fucose per ml.

(dimethyl sulfoxide) and nigericin (EtOH) did not stimulate the acrosome reaction when diluted 100× into sperm cultures (data not shown).

DISCUSSION

These studies provide further support for our hypothesis that the cannabinoid receptor in sea urchin sperm has a functional role in modulating the stimulation of the acrosome reaction by egg jelly. Anandamide, an endogenous ligand for the cannabinoid receptor in mammalian brain, blocks the triggering of the acrosome reaction and thereby reduces the fertilizing capacity of the sperm. These biological effects on sperm function during fertilization are similar to those produced by THC and other cannabinoids (1, 3, 4). The adverse effects of anandamide and THC on sperm fertilizing capacity and the acrosome reaction are reversible. Arachidonic acid, a likely precursor of anandamide (17), does not affect the triggering of the acrosome reaction at concentrations where this process is completely blocked by anandamide. As previously found with THC (4), anandamide does not block the acrosome reaction artificially initiated by ionophores. Anandamide appears to be slightly less potent than THC in affecting sperm function. Although the observed differences in potency are not statistically significant, they are in the right direction. Anandamide generally appears to be slightly less effective than THC in binding to cannabinoid receptors *in vitro* and in producing biological effects *in vivo* (sedation, hypothermia, analgesia) in mice (10, 11, 13, 25). Anandamide also shares with THC the capacity to produce biological effects outside the brain (10, 25).

The acrosome reaction triggered by egg jelly is induced by the opening of ligand-gated ion channels resulting in the net influx of Ca^{2+} and Na^+ and the net efflux of H^+ and K^+ (23, 24). Although anandamide and THC prevent the triggering of the acrosome reaction by egg jelly, they do not inhibit the acrosome reaction initiated by ionomycin and nigericin, which open Ca^{2+} and K^+ channels, respectively (ref. 4; this study). This suggests that anandamide and THC block the egg jelly-induced acrosome reaction by affecting an event(s) in the stimulus–secretion-coupling mechanism of the sperm prior to the opening of ion channels. These findings are consistent with observations on mammalian nerve cells that anandamide and various cannabinoids modulate the opening of calcium and potassium channels (12, 16, 26–29). The opening of calcium and potassium channels in sperm cells is part of the signal transduction mechanism associated with the stimulation of the acrosome reaction by egg jelly (8, 23, 24). Binding of anandamide and cannabinoids to their receptors in neural and nonneural cells also affects signal transduction by inhibiting the synthesis of cyclic AMP (11, 12, 26, 30). The production of cyclic AMP in the sperm head also is a component of the signal cascade stimulated by egg jelly to initiate the acrosome reaction (8). These signal pathways are potential targets for the biological actions of anandamide and the cannabinoids on sperm function.

Our findings raise the possibility that anandamide or a related compound may be the natural ligand for the cannabinoid receptor in sea urchin sperm. Anandamide may regulate sperm function prior to and/or during fertilization. If anandamide is present in seminal plasma then it may help to keep the sperm quiescent until they are diluted by sea water after spawning. Anandamide also may be produced by the egg during fertilization. Many sperm are likely to be in the vicinity of an unfertilized egg during the process of fertilization. As soon as the first sperm activates the egg, other sperm in the vicinity of the egg represent a potential hazard to normal development (reviewed in ref. 22). Penetration of the egg by more than one sperm (polyspermy) results in abnormal cleavage and death of the embryo. Secretion of the egg's cortical granules is one of the early responses of the egg to stimulation by the fertilizing sperm (22). Activation of phospholipase A_2 has been implicated in promoting cortical granule exocytosis and the release of arachidonic acid from membrane phospholipids in sea urchin eggs during fertilization (22, 31). Oxidative metabolites of arachidonic acid are formed by sea urchin eggs during fertilization (32–34) and have been implicated in helping to prevent polyspermy during fertilization (22, 35, 36). It is tempting to speculate that anandamide may be synthesized by the egg during the cortical reaction to prevent excess sperm in the vicinity from undergoing the acrosome reaction. If this hypothesis proves to be correct, then anandamide and its receptor in the sperm would constitute another component of the redundant set of defense mechanisms that act together to ensure that only one sperm penetrates the egg during fertilization (22).

The gene for the cannabinoid receptor in human brain also is expressed in the human testis (37). This finding taken together with the results of our studies on sea urchin sperm suggest that mature human sperm also might contain a cannabinoid receptor (1). If this hypothesis is correct, then our observations on the effects of anandamide and THC on the acrosome reaction and sperm fertilizing capacity in sea urchins could be relevant to human reproduction. In this connection it is interesting to note that other psychoactive drugs (cocaine, nicotine, opiates, etc.) directly affect fertilization in sea urchins (22). Human sperm contain cocaine and olfactory receptors (38, 39). These receptors, like the cannabinoid receptor in sea urchin sperm (1), may regulate normal sperm functions. This hypothesis is consistent with observations that sea urchin and/or mammalian sperm contain receptors for neurotransmitters (acetylcholine and catecholamines) and hormones (progesterone and natriuretic-like peptides). These receptors modulate normal sperm functions such as respiration, motility, chemotaxis, and the acrosome reaction (8, 40–45). The biological importance of these regulatory phenomena is reflected in the >70% conservation of amino acid sequences in the sea urchin sperm receptor for the egg jelly-derived peptide that stimulates sperm motility and receptors for natriuretic peptides in mammalian tissues (46).

Since the cannabinoid receptor has been found in sea urchin sperm (1) and in various neural and nonneural tissues in mammals (25–30, 37, 47, 48), it probably has an ancient origin in evolutionary history. This hypothesis also is supported by observations that THC affects brain function and social behavior in ants (49) as well as cyclic nucleotide metabolism and macromolecular synthesis in synchronously dividing protozoa (50, 51). Results of the present study suggest that anandamide also may have an ancient origin in evolutionary history. This possibility is consistent with the well-documented functional roles of metabolites derived from arachidonic acid in invertebrates and vertebrates (34). Our findings also suggest that reaction of anandamide and THC with the cannabinoid receptor may modulate response to stimulation in sea urchin sperm. This functional relationship appears to have been highly conserved during evolution since cannabinoids modulate responses to stimulation in a wide variety of cells—e.g., regulation of ion transport in pancreatic islet cells, lymphocytes, and nerve cells (16, 27–29, 52, 53, 60); inhibition of cyclic AMP production in protozoa, Sertoli cells, nerve cells, and fibroblasts transfected with the cloned cannabinoid receptor gene (11, 12, 30, 48, 51, 54); activation of phospholipase A_2 in various mammalian cells and sea urchin sperm (55, 56, 61); and inhibition of secretion by nerve cells, sea urchin sperm, and the pituitary gland (5, 57, 58). The human brain cannabinoid receptor also is expressed in human testis and leukocytes (37, 47). Another type of cannabinoid receptor is present in splenic macrophages but is not expressed in brain (25). Thus the "cannabinoid receptor" may comprise a family of related receptors. In this connection it would be of interest to rigorously compare the properties of the sea urchin sperm receptor with those of mammalian cannabinoid receptors in brain and peripheral organs. As is true for other kinds of receptors, more than one type of endogenous agonist for cannabinoid receptors may be present in animal tissues (10, 18). These findings suggest that cannabinoid receptors play a critically important and more general role in modulating cellular function than is associated with the psychoactive effects of cannabinoids. Sea urchin sperm are an ideal model system to determine how a cannabinoid receptor operates at the cellular level. Anandamide and the cannabinoids are useful probes to analyze the stimulation–secretion-coupling mechanism in sperm.

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1. Chang, M. C., Berkery, D., Schuel, R., Laychock, S. G., Zimmerman, A. M., Zimmerman, S. & Schuel, H. (1993) *Mol. Reprod. Dev.* **36**, 507–516.
2. Gaoni, Y. & Mechoulam, R. (1964) *J. Am. Chem. Soc.* **86**, 1946–1947.
3. Schuel, H., Schuel, R., Zimmerman, A. M. & Zimmerman, S. (1987) *Biochem. Cell Biol.* **65**, 130–136.
4. Schuel, H., Berkery, D., Schuel, R., Chang, M. C., Zimmerman, A. M. & Zimmerman, S. (1991) *Mol. Reprod. Dev.* **29**, 51–59.
5. Chang, M. C. & Schuel, H. (1991) *Mol. Reprod. Dev.* **29**, 60–71.
6. Dan, J., Ohori, Y. & Kushida, H. (1964) *J. Ultrastruct. Res.* **11**, 508–524.
7. SeGall, G. K. & Lennarz, W. J. (1979) *Dev. Biol.* **71**, 33–48.
8. Garbers, D. L. (1989) *Annu. Rev. Biochem.* **58**, 719–742.
9. Wassarman, P. M. (1987) *Science* **235**, 553–560.
10. Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. & Mechoulam, R. (1992) *Science* **258**, 1946–1949.
11. Vogel, Z., Barg, J., Levy, R., Saya, D., Heldman, E. & Mechoulam, R. (1993) *J. Neurochem.* **61**, 352–255.
12. Felder, C. C., Briley, E. M., Axelrod, J., Simpson, J. T., Mackie, K. & Devane, W. A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 7656–7660.
13. Fride, E. & Mechoulam, R. (1993) *Eur. J. Pharmacol.* **231**, 313–314.
14. Kuehl, F. A., Jacob, T. A., Ganley, O. H., Ormond, R. E. & Meissinger, M. A. P. (1957) *J. Am. Chem. Soc.* **79**, 5577–5578.
15. Bachur, N. R., Masek, K., Melmon, K. L. & Udenfriend, S. (1965) *J. Biol. Chem.* **240**, 1019–1024.
16. Johnson, D. E., Heald, S. L., Dally, R. D. & Janis, R. A. (1993) *Prostaglandins Leukotrienes Essent. Fatty Acids* **48**, 429–437.
17. Deutsch, D. G. & Chin, S. A. (1993) *Biochem. Pharmacol.* **46**, 791–796.

18. Hanus, L., Gopher, A., Almog, S. & Mechoulam, R. (1993) *J. Med. Chem.* **36**, 3032–3034.
19. Garrett, E. R. & Hunt, C. A. (1974) *J. Pharmacol. Sci.* **63**, 1056–1064.
20. Edwards, A. L. (1967) *Statistical Methods* (Holt–Reinhardt–Winston, New York), 2nd Ed.
21. Snedecore, G. W. & Cochran, W. G. (1980) *Statistical Methods* (Iowa State Univ. Press, Ames).
22. Schuel, H. (1984) *Biol. Bull.* **167**, 271–309.
23. Guerrero, A. & Darszon, A. (1989) *J. Biol. Chem.* **264**, 19593–19599.
24. Schackmann, R. W. (1989) in *The Cell Biology of Fertilization*, eds. Schatten, H. & Schatten, G. (Academic, San Diego), pp. 3–28.
25. Munro, S., Thomas, K. L. & Abu-Shaar, M. (1993) *Nature (London)* **365**, 61–65.
26. Felder, C. C., Veluz, J. S., Williams, H. L., Briley, E. M. & Matsuda, L. A. (1992) *Mol. Pharmacol.* **42**, 838–845.
27. Deadwyler, S. A., Hampson, R. E., Bennett, B. A., Edwards, T. A., Mu, J., Pacheco, M. A., Ward, S. J. & Childers, S. R. (1993) *Recept. Channels* **1**, 121–134.
28. Mackie, K. & Hille, B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3825–3829.
29. Mackie, K., Devane, W. A. & Hille, B. (1993) *Mol. Pharmacol.* **44**, 498–503.
30. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C. & Bonner, T. I. (1990) *Nature (London)* **346**, 561–564.
31. Ferguson, J. E. & Shen, S. S. (1984) *Gamete Res.* **9**, 329–338.
32. Perry, G. & Epel, D. (1985) *Dev. Biol.* **107**, 58–65.
33. Hawkins, D. J. & Brash, A. R. (1987) *J. Biol. Chem.* **262**, 7629–7634.
34. Stanley-Samuelson, D. W. (1991) *Am. J. Physiol.* **260**, R849–R853.
35. Schuel, H., Traeger, E., Schuel, R. & Boldt, J. (1984) *Gamete Res.* **10**, 9–19.
36. Schuel, H., Moss, R. & Schuel, R. (1985) *Gamete Res.* **11**, 41–50.
37. Gerard, C. M., Mollereau, C., Vassart, G. & Parmentier, M. (1991) *Biochem. J.* **279**, 129–134.
38. Yazigi, R. A., Odem, R. R. & Polakoski, K. L. (1991) *J. Am. Med. Assoc.* **266**, 1956–1959.
39. Parmentier, M., Libert, F., Schurmans, S., Schiffmann, S., Lefort, A., Eggerickx, D., Ledent, C., Mollereau, C., Gerard, C., Perret, J., Grootegoed, A. & Vassart, G. (1992) *Nature (London)* **355**, 453–455.
40. Cornett, L. E. & Meizel, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4954–4958.
41. Nelson, L., Young, M. J. & Gardner, M. E. (1980) *Life Sci.* **26**, 1739–1749.
42. Blackmore, P. F., Neulen, J., Lattanzio, F. & Beebe, S. J. (1991) *J. Biol. Chem.* **266**, 18655–18659.
43. Ralt, D., Goldenberg, M., Fetterolf, P., Thompson, D., Dor, J., Mashiach, S., Garbers, D. L. & Eisenbach, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2840–2844.
44. Young, R. J. & Laing, J. C. (1991) *Mol. Reprod. Dev.* **28**, 55–61.
45. Storey, B. T., Hourani, C. L. & Kim, J. B. (1992) *Mol. Reprod. Dev.* **32**, 41–50.
46. Schulz, S. (1992) *Biol. Bull.* **183**, 155–158.
47. Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., LeFur, G. & Casellas, P. (1993) *Eur. J. Biochem.* **214**, 173–180.
48. Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S. & Howlette, A. C. (1988) *Mol. Pharmacol.* **42**, 736–742.
49. Waser, P. (1993) in *Cannabis Physiopathology, Epidemiology, Detection*, eds. Nahas, G. C. & Latour, C. (CRC, Boca Raton, FL), pp. 359–367.
50. McClean, D. K. & Zimmerman, A. M. (1976) *Pharmacology* **14**, 307–321.
51. Zimmerman, S., Zimmerman, A. M. & Laurence, H. (1981) *Can. J. Biochem.* **59**, 489–493.
52. Laychock, S. G. (1991) *Exp. Clin. Endocrinol.* **10**, 63–71.
53. Yerba, M., Klein, T. W. & Friedman, H. (1992) *Life Sci.* **51**, 151–160.
54. Heindel, J. J. & Keith, W. B. (1989) *Toxicol. Appl. Pharmacol.* **101**, 124–134.
55. Burstein, S. (1992) in *Marijuana/Cannabinoids: Neurobiology and Neurophysiology*, eds. Murphy, L. & Bartke, A. (CRC, Boca Raton, FL), pp. 73–91.
56. Chang, M. C., Berkery, D., Laychock, S. G. & Schuel H. (1991) *Biochem. Pharmacol.* **42**, 899–904.
57. Roth, S. H. (1978) *Can. J. Physiol. Pharmacol.* **56**, 968–975.
58. Murphy, L. L., Newton, S. C., Dahli, J. & Chavez, D. (1991) *Pharmacol. Biochem. Behav.* **40**, 603–607.
59. Keller, S. H. & Vacquier, V. D. (1994) *Dev. Biol.* **162**, 304–312.
60. Nadler, V., Mechoulam, R. & Sokolovsky, M. (1993) *Brain Res.* **622**, 79–85.
61. Burstein, S., Budrow, J., Debatis, M., Hunter, S.-A. & Subramanian, A. (1994) *Biochem. Pharmacol.*, in press.