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The Newly Synthesized Linoleic Acid Derivative FR236924 Induces a Long-Lasting Facilitation of Hippocampal Neurotransmission by Targeting Nicotinic Acetylcholine Receptors

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Abstract—The newly synthesized linoleic acid derivative, FR236924, induces a long-lasting facilitation of hippocampal neurotransmission based on a persistent enhancement in the activity of presynaptic nicotinic ACh receptors via a PKC pathway and the ensuing increase in glutamate release, not only in vitro but in vivo at a low dosage (2 mg/kg, ip), which suggested the possibility of its use as a promising anti-dementia drug.

Essential fatty acids, such as arachidonic acid (**1**), linoleic acid (**2**), and linolenic acid (**3**) have long been thought to play important roles as raw materials for bioactive lipid mediators such as prostaglandins and/or leukotrienes. Therefore, few attempts to obtain synthetic essential fatty acid derivatives have been described by medicinal chemists. In recent years however, interesting biological activity themselves has been found in many areas, for example, activation of protein kinase C[1] and peroxisome proliferator-activated receptors.[2]

We found earlier that arachidonic acid (**1**), a *cis*-unsaturated free fatty acid, persistently enhances the activity of nicotinic acetylcholine (ACh) receptors via a protein kinase C (PKC) pathway.[3–5] The action of arachidonic acid on presynaptic nicotinic ACh receptors caused a marked increase in the release of the excitatory neurotransmitter glutamate, thereby leading to a long-lasting facilitation of hippocampal synaptic transmission, that resembles long-term potentiation (LTP), a cellular model of learning and memory.[5,6] A similar effect was also obtained with other *cis*-unsaturated free fatty acids, such as oleic, linoleic, and linolenic acid.[7,8] It is suggested from these results that *cis*-unsaturated free fatty acids could enhance cognitive function. Additionally, the effect of arachidonic acid on nicotinic ACh receptors was not inhibited by a lipoxygenase inhibitor or a cyclooxygenase inhibitor, suggesting that arachidonic acid itself, and not its bioactive metabolites such as prostaglandins and/or leukotrienes, potentiates nicotinic ACh receptor activity.[5] It is of major interest to know whether *cis*-unsaturated free fatty acids exert their actions in in vivo systems, since the free fatty acids can be promptly metabolized to generate the multi-bioactive metabolites and are thus decomposed. This prompted the present study to design FR236924 (**4**), a linoleic acid derivative without the double bonds, since they are known to be the key moieties in metabolic reactions in vivo, and to assess whether the compound exhibits activities similar to the original free fatty acids or not. The results of the present study demonstrate that FR236924 persistently potentiates nicotinic ACh receptor responses via a PKC pathway and induces an 'LTP-like' long-lasting facilitation of hippocampal neurotransmission. This suggests the possibility of use of FR236924 as a promising anti-dementia drug. Herein, we describe the design, synthesis, and biological activity of FR236924.

Design of FR236924

Our focus was to design compounds replacing the *cis*-double bonds by other biologically stable bioisosteres

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because of the reasons described above, and synthesized compounds bearing cyclopropane rings instead of the *cis*-double bonds[9] because of structurally similarity (Fig. 1).

We initially screened the compounds for enhancing action on nicotinic ACh receptor responses, using *Torpedo* nicotinic ACh receptors expressed in *Xenopus* oocytes (Table 1). Among the compounds examined, linoleic acid derivative (**4**, FR236924) and the oleic acid derivative (**8**) enhanced *Torpedo* nicotinic ACh receptor responses, with compound **4** showing the greater response. In contrast, the arachidonic acid derivative (**5**) and the linolenic acid derivative (**6**), inhibited the responses, whereas arachidonic acid and linolenic acid potentiated the *Torpedo* nicotinic ACh receptor response.[3,4,8] The mechanism underlying this inhibitory action of compounds **5** and **6** is presently unknown.

The synthesis of the cyclopropane derivatives used in this study is shown in Figure 2. Cyclomethylation onto the *cis*-double bond was carried out using diethylzinc and diiodomethane in dichloromethane and resulted in *cis*-cyclopropane derivatives as a mixture of diastereomers. Ester hydrolysis with 1 N LiOH at 60 °C in dioxane gave the desired compounds. After purification by chromatography on silica gel, they were used in the biological experiments.[10]

Biological Results and Discussion on FR236924

Of the neuronal nicotinic ACh receptor subunits cloned, α7 and α4β2 receptors are predominantly expressed in the brain.[11–15] α7 receptors are preferentially localized on presynaptic terminals and stimulate the release of the excitatory neurotransmitter glutamate in the hippocampus.[16,17] To examine the effect of FR236924 on neuronal nicotinic ACh receptors, α7 receptors were expressed in *Xenopus* oocytes. FR236924 (10 μM) induced a gradually-developing and persistent potentiation of α7 receptor responses, reaching 144±15% of original amplitude 25 min after treatment (Fig. 3A). The effect was inhibited by the selective PKC inhibitor, GF109203X (100 nM), suggesting that like linoleic acid,[8] FR236924 potentiates nicotinic ACh receptor responses via a PKC pathway (Fig. 3B).

If FR236924 persistently enhances α7 receptor activity, then it could facilitate hippocampal neurotransmission by enhancing presynaptic glutamate release. To address this point, we monitored population spikes (PSs) in the dentate gyrus of rat hippocampal slices. As expected, FR236924 facilitated hippocampal neurotransmission in a bell-shaped dose-dependent manner at concentrations ranging from 10 nM to 10 μM (Fig. 3C). The compound sustained the facilitation more than 60 min after 10-min treatment. The maximal effect was obtained with 100 nM FR236924, and the potentiation reached 227±6% of basal PS amplitude 60 min after treatment (Fig. 3C). The facilitatory action was inhibited by α-bungarotoxin

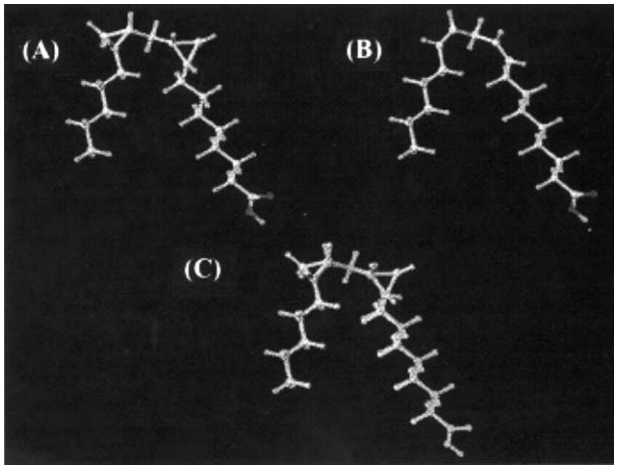

Figure 1. Structural similarity between linoleic acid and FR236924 (**4**). (a) A molecular mechanics (MM) structure of **4** [(9*S*, 10*R*, 12*R*, 13*S*)-FR236924 was presented in this figure. Similar results were obtained on study of the other three isomers], (b) that of linoleic acid, and (c) a superimposed structure of **4**, and linoleic acid. Structure of linoleic acid was obtained by the Maximin2 force field of SYBYL version 6.7 using a linear conformation as the starting structure, and was thought to be a one of its local minimum structure (no conformation search study was performed). Structure of **4** was obtained in the way that that of **4** was superimposed on the linoleic acid structure using MULTIFIT function of SYBYL version 6.7.

Table 1. The effect of *cis*-unsaturated free fatty acids and their derivatives (10 μM) on Torpedo nicotinic ACh receptor responses

Structure	Ach receptor responses (% of original amplitude)	
	0 min	10 min
Arachidonic acid (**1**)	78±5	139±4
Linoleic acid (**2**)	72±5	175±16
Linolenic acid (**3**)	77±7	272±7
4 (FR236924)	122±4	156±16
5	88±8	78±5
6	87±5	69±9
Oleic acid (**7**)	108±5	139±6
8	117±7	124±16

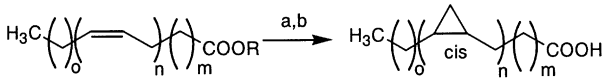

Figure 2. Synthesis of cyclopropane derivatives. Reagents and conditions: (a) Et_2Zn, CH_2I_2/CH_2Cl_2, −5 to 0 °C, (b) 1 N LiOH/dioxane, 60 °C.

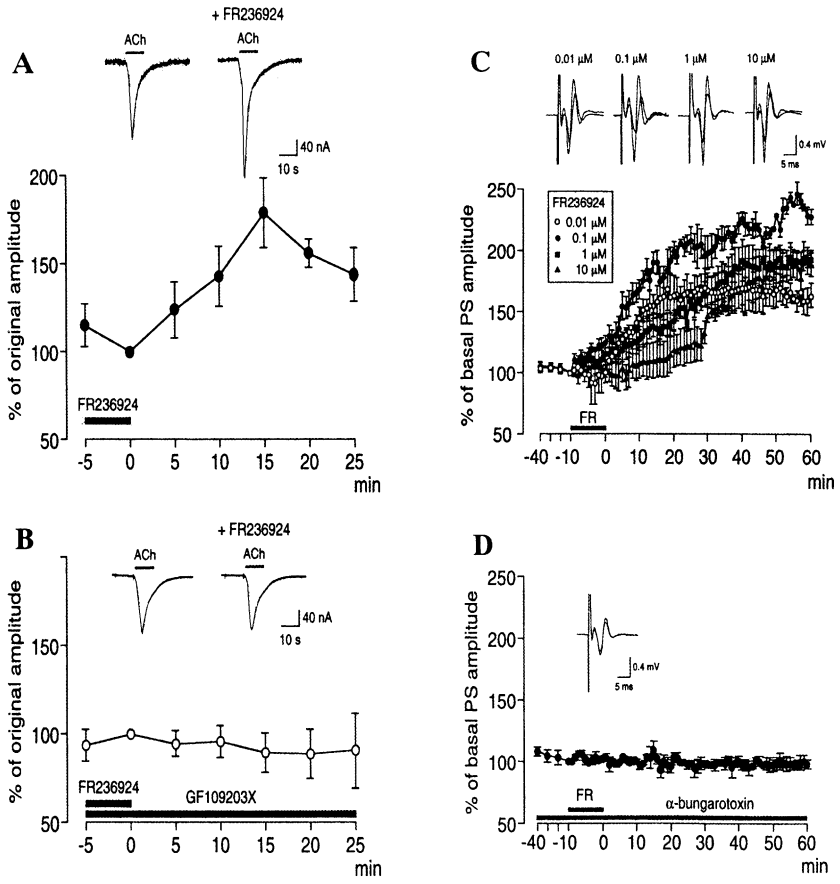

Figure 3. (A, B) Effect of FR236924 on whole-cell membrane currents through α7 receptor channels. (C, D) Effect of FR236924 on PSs in rat hippocampal slices. (A) ACh (100 μM) was applied to an oocyte before and after 5-min treatment with FR236924 (10 μM) with 5-min intervals. (B) ACh (100 μM) was applied to an oocyte before and after 5-min treatment with FR236924 (10 μM) with 5-min intervals in the presence of GF109203X (100 nM). The holding potential was −60 mV. (C) PSs were monitored from the granular cell layer of rat hippocampal slices before and after 10-min treatment with FR236924 at the concentrations indicated. (D) PSs were recorded before and after 10-min treatment with FR236924 (100 nM) in the presence of α-bungarotoxin (50 nM).

(50 nM), an inhibitor of α7 receptors (Fig. 3D). Taken together, FR236924 appears to induce an 'LTP-like' long-lasting facilitation of hippocampal neurotransmission by enhancing nicotinic ACh receptor activity.

To finally examine the in vivo effect of FR236924, we monitored PSs in the dentate gyrus of the intact rat hippocampus. Before this examination, we measured the concentration of FR236924 in blood after ip and po administration.[18] FR236924 was detected in blood at more than 0.5 μg/mL (∼1.8 μM), that is 180 times greater than that of the maximum effective concentration (100 nM). These results raised our hopes that ip administration of FR236924 would be effective in intact rat, as well as in the in vitro study.

As expected, FR236924 (2 mg/kg, ∼6 μM, ip) facilitated hippocampal neurotransmission, the effect being evident 120 min after injection (186 ± 15% of basal PS amplitude) (Fig. 4). This would imply that the stable facilitatory action of FR235924 on hippocampal neurotransmission is still obtained with the in vivo system.

Neuronal nicotinic ACh receptors as well as muscarinic ACh receptors are constituted of the cholinergic systems, and are rich in the limbic systems including the

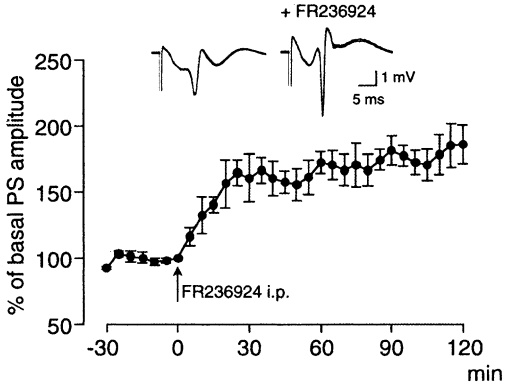

Figure 4. Effect of FR236924 on PSs in the intact rat hippocampus. PSs were recorded before and after injection with FR236924 (2 mg/kg, ip). In the graph, each point represents the mean (±SEM) percent of basal PS amplitude (0 min) ($n = 6$). Illustrated PSs were recorded 0 and 120 min after injection with FR236924.

hippocampus, a center of cognitive function.[19] A consistent finding is that cholinergic systems are disrupted in the Alzheimer brain.[20–22] In addition, the number of nicotinic ACh receptors decreases in the aging brain.[23] Nicotinic ACh receptors, thus, are likely to play a significant role in cognitive functions. We earlier found that perforant path LTP and Schaffer-collateral LTP,

which express in an *N*-methyl-D-aspartate (NMDA) receptor-dependent manner,[24,25] were induced by activating nicotinic ACh receptors, independently of NMDA receptors.[26] This suggests that nicotinic ACh receptors are a downstream target of the NMDA receptor signal in the expression of LTPs.[26]

An important question is: what signal is involved in the LTPs? The answer may be that *cis*-unsaturated free fatty acids may mediate it. High frequency stimulation to induce LTP causes a huge increase in the release of glutamate followed by high activation of NMDA receptors, thereby producing *cis*-unsaturated free fatty acids, such as arachidonic, oleic, linoleic, and linolenic acid, which in turn, persistently enhance presynaptic nicotinic ACh receptors, leading to a long-lasting facilitation of hippocampal synaptic transmission.[5,6] *Cis*-unsaturated free fatty acids, therefore, may serve as retrograde messengers in NMDA receptor-dependent LTP. This raises the possibility that compounds targeting nicotinic ACh receptors, like *cis*-unsaturated free fatty acids, can enhance learning and memory; that is, amelioration of dementia, such as Alzheimer disease and senile memory deficits. In the present study, FR236924, a linoleic acid derivative without unstable *cis*-double bonds, induced an 'LTP-like' long-lasting facilitation of hippocampal neurotransmission in both in vitro and in vivo systems by targeting nicotinic ACh receptors. This gives us hope that FR236924 could be a novel and promising drug against a variety of forms of dementia. Further pharmacological and physiological studies on FR236924 in vitro and in vivo will be reported in the future.

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9. We have also synthesized compounds having phenyl and hetero rings instead of the *cis*-double bonds, and compounds with a tetrazole ring, esters, amides, and alcohols, aiming for bioisosteres of the caroboxylic acid moiety, resulting in reduction of the bioactivities (data will be reported in the future). These cyclropropane derivatives are racemic mixture, and we have not succeeded in separation of these isomers by HPLC yet.
10. Representive procedure for synthesis of compounds studied in this work: To a solution of linoleic acid methyl ester (2.5 g, 8.49 mmol) in dichloromethane (CH_2Cl_2, 50 mL), a 0.99 M *n*-hexane solution of diethyl zinc (103 mL, 101.88 mmol) was added under a N_2 atmosphere, with cooling with an ice-water bath (−5 to 0 °C), and then stirred for 1 h. Diiodomethane (16.4 mL, 203.76 mmol) was added thereto, and the mixture was stirred at ambient temperature overnight. After removal of solvent by evaporation, the objective was extracted by ethyl acetate (EA), was purified by chromatography on silica gel (eluted with 1% EA in *n*-hexane) to give 8-(2-((2-pentylcyclopropan-1-yl)methyl)cyclopropyl)octanoic acid methyl ester (2.81 g, 102%) as an oil. A mixture of this ester (4.89 g, 15.16 mmol), 1 N LiOH (33.4 mL, 33.4 mmol), and dioxane (33 mL) was stirred at 60 °C overnight. The objective was extracted by EA, was purified by chromatography on silica gel (eluted with 20% EA in *n*-hexane). Fractions including the object compound were collected and evaporated in vacuo to give **4** as an oil (4.01 g, 85.8%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: −0.30 (m, 2H), 0.6–1.7 (m, 30H), 1.64 (t, 2H, J = 7.2 Hz), 2.31 (t, 2H, J = 7.2 Hz). Anal. calcd for CHO: C, 77.87; H, 11.76. Found: C, 78.54; H, 12.11.
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18. We measured the concentration of FR236924 in blood after ip and po administration of a high dosage of FR236924 (100 mg/kg), because it is difficult to detect low concentrations of FR236924 in blood because it has no useful UV or fluorescent absorption, and affords a weak mass spectrometer (MS) ion peak. Therefore, we detected it by MS after conversion to Ser derivative by standard amide coupling reaction.
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