Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 447–449

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Evaluation of Novel Amides of Polyunsaturated Fatty Acids with Dopamine

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Received 1 August 2000; accepted 22 November 2000

Abstract—New amides of different fatty acids from the C18, C20, and C22 series with dopamine were synthesized. Pharmacological characterization in binding assays with rat brain membrane preparations and in the 'tetrad' of cannabinoid behavioral tests showed that, for these compounds, cannabinoid-like activity was dependent on the fatty acid moiety. Our data demonstrate that polyenoic fatty acid amides with dopamine comprise a new family of synthetic cannabimimetics. © 2001 Published by Elsevier Science Ltd.

Recent biochemical and pharmacological studies have led to the characterization of different fatty acid amides as a new family of biologically active lipids.[1,2] Oleamide (amide of oleic acid) is an endogenous fatty acid primary amide that accumulates in the cerebrospinal fluid under conditions of sleep deprivation and induces physiological sleep in animals[3] and could be referred as a prototypical member of this family.[1] Anandamide (*N*-arachidonoylethanolamine) and other ethanolamides of polyunsaturated fatty acids have been reported to displace radioactive cannabinoid ligands from specific binding sites in brain membranes,[4] to inhibit adenylyl cyclase[5] and *N*-type calcium channels[6] and to produce hypothermia, catalepsy, analgesia and hypoactivity (the latter four tests known as the cannabinoid 'tetrad').[7–10] Studies of the structure–activity relationships of anandamide and oleamide analogues revealed the importance of their fatty acid moieties for biological activity.[11,12] Recently we demonstrated that *N*-arachidonoyldopamine is a potent effector in different biological models.[13] Here we describe the synthesis of amides of different polyunsaturated fatty acids (PUFAs) from C18–C22 families with dopamine (DA) and demonstrate for the first time that these compounds produce cannabinoid-like effects in vivo.

All PUFAs used, except arachidonic acid (commercial product), were isolated from natural sources or were prepared from available fatty acids by partial chemical synthesis using C2-elongation approach.[14] DA amides of PUFAs (Fig. 1) were prepared by the 'mixed anhydride' technique with average yields of 55–65%.[15] We tested other methods of amide bond formation

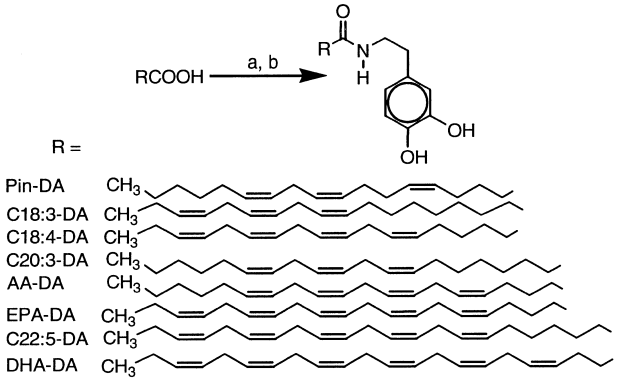

Figure 1. Synthesis of dopamine derivatives of PUFAs. (a) *iso*-Butylchloroformate, acetonitrile, NEt_3, +4°C, 1 h; (b) dopamine hydrochloride, DMF, NEt_3, +4 °C, 18 h.

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0960-894X/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0960-894X(00)00689-2

Table 1. Influence of compounds **1–8** and anandamide on the immobility time in the 'ring' test, on the rectal temperature, on the latency time in the 'hot-plate' test and activity in the binding assay

Compound	Ring (%)	Rectal temperature (°C)		Hot plate (%)	K_i (μM)
		5 min	10 min		
Pin-DA	528[a]	0.5 (±0.22)	0.4 (±0.14)	162	1.72
C18:3-DA	499[a]	0.32 (±0.3)	0.68 (±0.3)	114	3.13
C18:4-DA	570[a]	1.45 (±0.26)	1.67 (±0.3)	120	2.43
C20:3-DA	693[a]	0.43 (±0.06)	0.7 (±0.1)	129	nt
AA-DA	500[a]	1.35 (±0.38)	1.27 (±0.46)	222[b]	0.38
EPA-DA	658[a]	1.64 (±0.4)	nt	135[b]	0.62
C22:5-DA	610[a]	1.35 (±0.17)	1.82 (±0.3)	144[b]	1.37
DHA-DA	674[a]	0.9 (±0.14)	1.1 (±0.17)	296[b]	nt
Anandamide	750[a]	1.93 (±0.15)	2.53 (±0.15)	165[b]	0.8

Data for 'ring' and 'hot-plate' tests are given as % of control. The decrease in rectal temperature 5 and 10 min after ip injection is represented as difference in temperature before and after the treatment. Binding assay were performed with 0.4 nM of [^{3}H]SR141716A (Amersham 60 Ci/mmol) and whole rat brain membrane preparations in the presence of phenylmethylsulphonylfluoride (0.1 mM), following a procedure previously described.[21] Specific binding was calculated in the presence of 10 μM SR141716A.
[a]$p < 0.01$.
[b]$p < 0.05$ (Student t-test); nt, not tested.

including: (1) reaction of PUFA acyl chlorides and acyl fluorides, prepared from the corresponding PUFA by treatment with either $SOCl_2$ (10 equiv, benzene, 50 °C, 2 h) or cyanuric fluoride[16] (1 equiv, Py 1 equiv, CH_3CN, rt, 60 min), with DA hydrochloride (NEt_3 1.2 equiv, $CHCl_3$, +4 °C, 18 h), or (2) reaction of PUFA imidazolides, prepared from PUFA and 1,1-carbonyldiimidazole (1.2 equiv, CH_3CN, 22 °C, 1 h), with DA hydrochloride (1 equiv, NEt_3 1 equiv, DMF, +4 °C, 18 h). All these methods were less satisfactory (yields < 40%) and the reactions were accompanied by the formation of the corresponding PUFA imides as major by-products.[17]

To assess the biological activity of the synthesized compounds in vivo we performed the tetrad of tests ('open field', 'ring', 'hot-plate', and 'hypothermia' tests), which is often used for evaluation of the cannabimimetic properties of a chemical substance.[19] The ability of a compound to produce catalepsy, hypothermia, and analgesia and to inhibit locomotion is considered as strong evidence of its cannabimimetic action.[20]

In the 'ring' test[8] all substances produced catalepsy, the time of immobility on the ring being 5–7 times higher than that of the control group (Table 1). Activities of the compounds in this test decreased in the following order: C20:3-DA >DHA-DA≈EPA-DA >C22:5-DA >C18:4-DA >Pin-DA≈AA-DA≈C18:3-DA > >control.

The most active compounds inducing analgesia in the 'hot-plate' test[10] were AA-DA, EPA-DA, and C22:5-DA with the maximal effect on the 6th minute. Weak analgesic effects were also observed with other substances but the difference between the test and the control groups was not statistically significant (Table 1). Unlike the other amides, DHA-DA produced the maximal analgesic effect on the 11th minute.

Several DA amides, namely C18:4-DA, AA-DA, EPA-DA, C22:5-DA and DHA-DA reduced rectal temperature by more than 1 °C and were considered as active in the 'hypothermia' test (Table 1).

Three of the amides showing the highest scores in three tests mentioned above (i.e., AA-DA, EPA-DA, and DHA-DA) were assayed also in the 'open field' test.[9] AA-DA at 5 and 10 mg/kg doses potently decreased locomotor activity and completely inhibited grooming behavior (Fig. 2). The nonselective dopamine D_2-receptor antagonist haloperidol (0.05 mg/kg) did not significantly antagonize the effects of AA-DA on locomotor (Fig. 2a) and vertical activities (data not shown) while it completely abolished AA-DA-induced inhibition of grooming activity (Fig. 2b). Locomotor

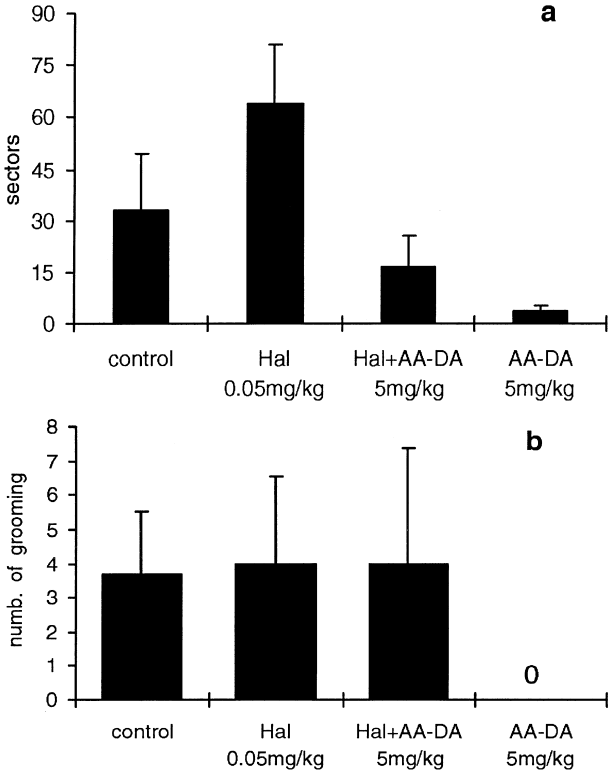

Figure 2. The influence of AA-DA (5 mg/kg) and haloperidol (Hal, 0.05 mg/kg) on the locomotor activity (a) and grooming behavior (b) of mice in the 'open field' test was registered during 15 min as number of crossed sectors or grooming, respectively.

activity was also reduced after administration of DHA-DA and EPA-DA at a dose of 10 mg/kg (15%, $P < 0.01$ and 42%, $P < 0.05$ of the control, respectively) while grooming behavior was again completely suppressed.

To elucidate whether the cannabimimetic properties of PUFA-DA are mediated via CB_1 cannabinoid receptors, experiments on the displacement of CB_1-antagonist [^{3}H]SR141716A from rat brain membranes were performed as previously described.[21] Among the compounds tested, AA-DA and EPA-DA were the most active (Table 1).[22]

Our data demonstrate that amides of PUFAs with dopamine possess pronounced cannabimimetic properties, especially if the fatty acid moiety contains four or more double bonds. This suggests that these compounds should be added to the family of CB_1-cannabinoid receptor agonists. The interaction of these molecules with the dopaminergic system as well as other biochemical properties of PUFA-DA were described elsewhere. [23]

Acknowledgements

Partial support by INTAS (No. 96-0987), Russian Foundation for Basic Research (No. 99-04-48514 and No. 00-04-81087), bilateral CNR-RAS support (to MYB No. 09) and MURST (to VDM, No. 3933) is acknowledged.

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