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Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent

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Anandamide (arachidonylethanolamide) is a brain constituent which binds to the cannabinoid receptor. We now report the first in vivo examination of this ligand. Anandamide administered i.p. in mice, caused lowering of activity in an immobility and in an open field test, and produced hypothermia and analgesia. These effects parallel those caused by psychotropic cannabinoids.

Cannabinoids; Tetrahydrocannabinol; Marihuana

Recently we described the isolation from porcine brain, the structure elucidation and the synthesis of arachidonylethanolamide (anandamide), a novel brain constituent that binds to the cannabinoid receptor and inhibits the electrically evoked twitch response of the vas deferens (Devane et al., 1992). So far no pharmacological data on anandamide in vivo have been reported. We now report that in several tests in mice the activity of anandamide parallels, to a large extent, the activity of Δ^8-tetrahydrocannabinol (Δ^8-THC).

Anandamide was prepared as previously described (Devane et al., 1992). It was dissolved in Emulphor 620 (GAF Corp. Linden, NJ) and ethanol; then saline was added so that in the final formulation their ratios were 1:1:18. It was given intraperitoneally (i.p.) to Sabra strain female mice (6–7 weeks old). The injection volume was 0.1 ml/10 g mouse.

The cannabinoids produce a unique syndrome of behavioral effects in animals: at low doses a mixture of depressant and stimulatory effects is observed, while at higher doses central depression predominates (Dewey, 1986; Pertwee, 1988). We examined anandamide for behavioral effects using tests frequently employed in this connection: the ring immobility (catalepsy) test, which measures the percent of time over 4 min mice remain motionless on a ring (5.5 cm diameter) (Pertwee, 1972) and the open field test, which measures horizontal (locomotor) and vertical (rearing) activity. Hypothermia (Δ°C) and antinociception (hot plate latency) were also measured. Martin et al. (1991) have shown that, when taken individually, these assays are not selective for any particular class of compounds. However this tetrade of tests, evaluated together 'has proven to be highly predictive of cannabinoids'. Each group of animals was evaluated consecutively in all four tests as it has been shown that the procedure of multiple testing of cannabinoids does not produce results significantly different from those observed when groups are evaluated in only one of the procedures (Compton et al., 1992). The data were analyzed by analysis of variance. Individual comparisons were done by Fisher's protected least significance difference test ($P < 0.05$).

The results of the above tests are presented in the table 1. They are compared to those obtained with Δ^8-THC (20 mg/kg). The effects of anandamide were measured at 4, 10, 15, 25, 45 and 90 min after injection. Peak activity on i.p. administration was observed for anandamide after about 10 min (10 mg/kg) and for Δ^8-THC after about 30–60 min. Hence the tests were initiated at 10 and 30 min respectively. The control animals were given vehicle only. The behavior of these animals did not differ significantly from that of uninjected mice and hence the data for these two groups were pooled ($n = 9$).

At a low dose (0.01 mg/kg) anandamide caused significant stimulation in the open field tests, but no significant changes were observed in the other tests (data not shown). At higher doses (see table 1) anan-

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TABLE 1

Pharmacological activity of anandamide administered i.p. to Sabra strain mice. For experimental details see text. The results are means ± S.E. where the asterisk represents a significant difference from the control (* $P < 0.05$). Each animal was tested in all tests. The order of testing was open field; ring test; body temperature; hot plate.

Dose range (mg/kg)	0	1	3	5	10	20	Δ^8-THC (20)
N of animals	9	7	7	7	7	5	5
Ring test [a]	21.2 ± 5.0	9.2 ± 2.9	16.4 ± 6.5	22.4 ± 3.0	45.8 ± 6.8 *	54.2 ± 11 *	69.6 ± 4.2 *
Open field							
ambulation [b]	115 ± 17	140 ± 25	65 ± 10 *	65 ± 12 *	32 ± 8 *	33 ± 16 *	60 ± 9 *
Rearing [c]	59 ± 9.3	54 ± 11.7	31 ± 7.1 *	27 ± 7.3 *	6 ± 3.3 *	10 ± 7.7 *	8 ± 3.3 *
Hypothermia [d]	0	0.08 ± 0.38	−0.78 ± 0.25 *	−0.44 ± 0.19	−1.62 ± 0.14 *	−2.63 ± 0.46 *	−3.15 ± 0.26 *
Hot plate [e]	7.4 ± 0.8	6.5 ± 1.2	10.9 ± 1.5	16.4 ± 2.8 *	18.4 ± 3.6 *	12.6 ± 2.3	35.6 ± 5.7 *

[a] Percentage time over 4 min spent immobile on ring. [b] Locomotion was measured by the number of squares crossed during an 8-min exposure immediately after entrance into the open field (20 × 30 cm divided into 12 squares of equal size). [c] Number of rearings during the same 8-min exposure. [d] Body temperature was measured with a Yellow Springs Instruments Co. telethermometer immediately before injection and then after completion of the ring test. The difference (°C) between the two measurements was compared to that of the control group, which was defined as zero. [e] Time (in s) until first hind paw lick. Temperature of hot plate, 54C°. The animals were removed from the plate after 45 s.

damide produced a significant depression in the open field tests, an increase in the time spent on the ring, hypothermia and analgesia. By comparison with the effects produced at a dose level of 100 mg/kg, we determined that at 20 mg/kg anandamide had reached its maximal effect, except in the ring test. In the latter the percent catalepsy reached at 100 mg/kg was 79.2 ± 3.0.

The results presented in table 1 are for Sabra strain female mice. We have obtained similar, significant results with Sabra male mice and with C57Bl (male and female) mice (10 mg/kg).

While numerous cannabinoids, both plant-derived and synthetic, have been evaluated in the above four tests, our results are the first ones recorded with a brain constituent. As anandamide has been shown to bind to the cannabinoid receptor and to inhibit the twitch response of the vas deferens, the above results provide further evidence that anandamide is an 'endogenous cannabimimetic'.

It is generally accepted that the action of cannabinoids is predominently central (Pertwee, 1988). Therefore the observations now reported indicate that anandamide rapidly crosses the blood-brain barrier.

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