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Clavulanic acid stimulates sexual behaviour in male rats

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ABSTRACT

Sexual behaviour in rats can be used to predict putative effects on human sexual behaviour. Anecdotic reports exist, that the beta-lactamase inhibitor, clavulanic acid exerts sexual stimulating activities in monkeys. To characterize these pro-sexual activities, clavulanic acid was tested in three doses and compared to one dose of a sexually inhibitory dose of the selective serotonin reuptake inhibitor, paroxetine, in sexually-experienced male rats, selected for a moderate level of sexual performance in a standard 30-min test with an oestrus female. After acute administration, clavulanic acid had minor sexual stimulating effects at the highest dose in the number of intromissions and in the first ejaculation series. After sub-chronic 7-days treatment, clavulanic acid increased the number of ejaculations at all three doses and reduced the number of intromissions in the 1st series at the highest dose. After chronic 14 days treatment, a similar but stronger pro-sexual profile was observed. The sexual side effects of paroxetine were as expected, including slight sexual inhibitory effects after acute administration, but somewhat stronger overall inhibitory effects after 7 and 14-days pretreatment, particularly notable in the decreasing number of animals contributing to the 2nd ejaculation series, which was even stronger after 14-days treatment. One week after cessation of treatment, the paroxetine group had completely recovered, whereas the highest dose-group of clavulanic acid still showed some pro-sexual effects. This remarkable pro-sexual activity of clavulanic acid cannot readily be explained by its mechanism of action as a beta-lactamase inhibitor but could be due to unexpected central activity of the compound.

1. Introduction

In humans, the area of sexual disturbances and their treatment has become a field of intense research particularly after the emergence of phosphodiesterase type 5 inhibitors for erectile dysfunction (Aversa et al., 2006). Moreover, quite a number of psychotropic drugs exert unwanted sexual side effects (Waldinger et al., 2002; Rosenzweig-Lipson et al., 2007). Notoriously, this holds for many antidepressants, and particularly selective serotonin reuptake inhibitors (SSRIs), where sexual disturbances including libido, erection and orgasm problems belong to the main complaints (Rosen et al., 1999; Balon, 2006). Although depression on itself is associated with these same problems, it is highly desirable to develop drugs without these cumbersome sexual side effects. Although it is desirable to develop new psychotropics, including antidepressants without sexual side effects, drugs that treat non-drug related sexual dysfunctions would also be needed.

Currently, such drugs are not readily available although some developments are in progress, both for male (Rosenzweig-Lipson et al., 2007) and female sexual dysfunction (Brown et al., 2007; Nurnberg et al., 2008). Therefore, research in new drugs aiming at improvement of sexual function, physiology and behaviour is absolutely necessary. Animal models of sexual behaviour are considered useful in predicting effects of drugs in humans (Ågmo and Ellingsen, 2003) and are used to study the effects of various drugs. Antidepressants and particularly SSRIs, like in humans have sexual-inhibiting effects, but only after chronic administration (Mos et al., 1999; Waldinger et al., 2001; de Jong et al., 2005; Chan et al., 2008).

We have found compelling evidence (Olivier et al., 2006; Pattij et al., 2005; Chan et al., 2008) that male rats display 'endophenotypes' with regard to sexual performance: some animals are sluggish copulators (low number of ejaculations), some are normal (2–3 ejaculations per test) and some are rapid (4–5 ejaculations/test). Our testing hypothesis tries to exploit these endophenotypes. In the present study we used the 'normally' performing rats (around 2–3 ejaculations/test) to be able to observe potential facilitating effects of the drugs on sexual activities.

Using this strategy we tested the compound clavulanic acid. Clavulanic acid is produced by *Streptomyces clavuligenus* with a chemical structure similar to some β-lactamines, e.g. penicillin and is used clinically and veterinary as a beta-lactamase inhibitor, often in combination with penicillin, e.g. amoxicillin. Clavulanic acid by itself is orally active and stable. The cerebrospinal fluid/plasma ratio in humans

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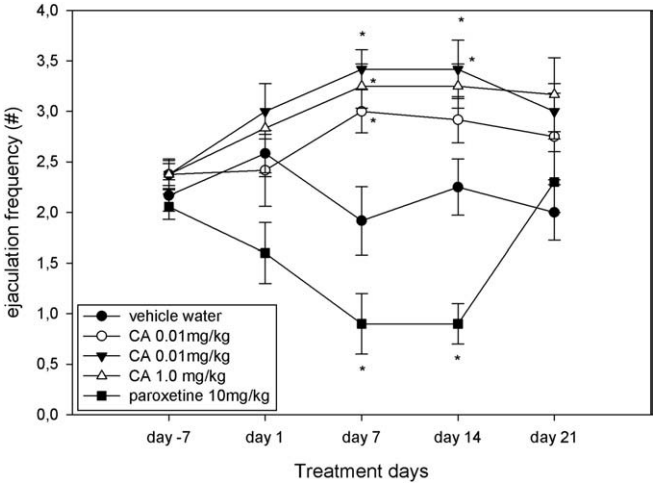

Fig. 1. Effects of vehicle, clavulanic acid (CA) and paroxetine on the number of ejaculations in sexual behaviour of male rats. Data (mean ± S.E.M.) are given for the last training day (day −7), after acute, sub-chronic, and chronic (Days 1, 7, and 14, respectively) treatment and one week after cessation of treatment (Day 21). * = indicates significant difference ($P<0.05$) from vehicle at the corresponding day.

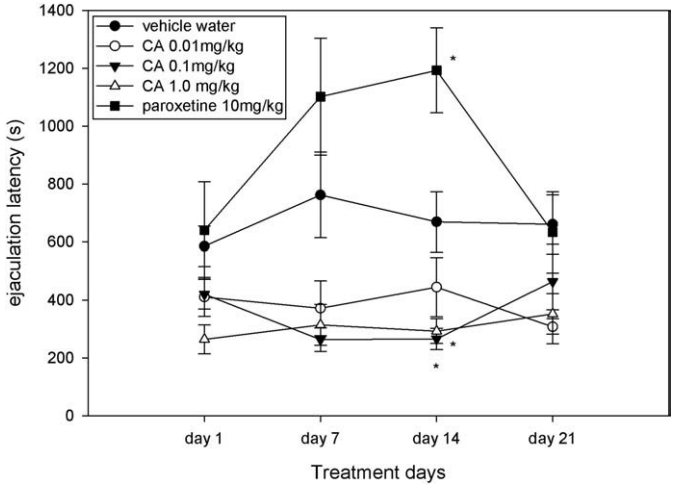

Fig. 2. Effects of vehicle, clavulanic acid (CA) and paroxetine on the latency to the first ejaculation in sexual behaviour of male rats. Data (mean ± S.E.M.) are given for the last training day (day −7), after acute, sub-chronic, and chronic (Days 1, 7, and 14, respectively) treatment and one week after cessation of treatment (Day 21). * = indicates significant difference ($P<0.05$) from vehicle at the corresponding day.

is around 0.25, indicating that clavulanic acid readily passes the blood-brain barrier (Nakagawa et al., 1994). While testing anti-anxiety effect of clavulanic acid in non-human primates (cotton-top tamarin), it was discovered that it increased sexual arousal as indicated by the increased rate of penile erections (unpublished findings by Rexahn).

The assumption in the present experiment is that this molecule potentially would stimulate sexual performance in male rats. As a reference compound we tested a selected dose of paroxetine (10 mg/kg) as this dose has reliably and repeatedly been shown to inhibit sexual activities after chronic, but not acute administration (Chan et al., 2008).

Drugs were administered for 14 days, and effects were measured acutely (Day 1), after 7 Days (Day 7) and after 14 Days (Day 14) of administration. Moreover, one week after cessation of treatment (Day 21) one extra sexual test (no treatment) was performed to study the potential 'after' effects of all chronic treatments.

2. Materials and methods

Male and female Wistar rats of approx. 8 weeks old were derived from Harlan (The Netherlands) and were group-housed. The day–night schedule was reversed (Lights off from 6:00AM till 6:00PM). After habituation to the lighting schedule, males were trained once weekly for 4 consecutive weeks without injection against an oestrus female in a 30-min test in an observation cage (30*40*60 cm) with a Plexiglas front. Females were brought into behavioural oestrus by injecting 50 μg estradiol benzoate (dissolved in sesame oil) in the nape 36 h prior to the test. The number of ejaculations over the last training test (#4) was used to designate animals either as low, medium (normal) or high performers. In the present experiments, drugs were tested on 'normal performing' animals ($n=60$) with on average 2 ejaculations. After this training period drug testing started and the 60 animals were randomized over the 5 experimental groups.

The following experiment was performed on 5 groups of 12 rats each: Group 1: Animals were orally injected once daily with the vehicle (distilled water) during 14 days. Groups 2, 3 and 4: clavulanic acid treatment in doses of 0.01 mg/kg (Group 2), 0.1 mg/kg (Group 3) and 1 mg/kg (Group 4). Group 5: Animals were orally injected once daily with 10 mg/kg paroxetine (suspended in distilled water) during 14 days.

Clavulanic acid was dissolved in distilled water and orally administered. On non-experimental testing days, all assigned treatments were injected between 10AM and 4PM. On the experimental days (Days 1, 7 and 14) animals were injected with the assigned treatment 60 min before testing. On Day 21, one week after cessation of treatment another sexual behaviour test was performed (post-

Table 1
Effects of chronic administration of clavulanic acid and paroxetine on sexual behaviour in male Wistar rats — acute treatment Day 1.

Parameter	Vehicle	Paroxetine	CA 0.01 mg/kg	CA 0.1 mg/kg	CA 1 mg/kg	ANOVA sig.
EF (#)	2.6 ± 0.2	1.6 ± 0.3	2.4 ± 0.4	3.0 ± 0.3	2.8 ± 0.4	0.040
LM1 (s)	61.8 ± 25.1	258.9 ± 138.9	23.8 ± 8.2	22.1 ± 13.3	76.3 ± 36.1	0.076
LI1(s)	26.4 ± 9.0	403.2[a] ± 185.9	16.1 ± 3.2	27.1 ± 7.3	49.3 ± 19.0	0.006
MF1 (#)	16.1 ± 3.4	14.7 ± 4.8	12.1 ± 4.4	8.3 ± 1.3	9.8 ± 3.2	0.539
IF1 (#)	12.3 ± 1.4	6.1[a] ± 0.8	9.3 ± 0.8	8.9 ± 0.8	7.9[a] ± 1.1	0.002
LE1 (s)	585.1 ± 69.8	640.1 ± 167.4	410.7 ± 67.2	420.2 ± 51.7	264.2 ± 49.5	0.062
PEL1 (s)	284.5 ± 18.8	802.2 ± 213.2	262.9 ± 20.3	242.6 ± 27.2	246.3 ± 20.8	0.042
CE1 (%)	47.5 ± 6.0	39.1 ± 8.3	54.0 ± 5.7	53.3 ± 4.7	56.8 ± 6.8	0.318
MF2 (#)	12.1 ± 3.3	18.4 ± 8.0	9.3 ± 1.6	7.0 ± 1.7	6.2 ± 1.5	0.121
IF2 (#)	3.9 ± 0.4	4.4 ± 0.6	5.0 ± 1.3	3.0 ± 0.4	4.0 ± 0.4	0.347
LE2 (s)	258.7 ± 43.4	375.5 ± 97.5	336.6 ± 96.8	248.9 ± 48.4	184.8 ± 32.3	0.315
PEL2 (s)	355.2 ± 15.7	409.8 ± 10.3	364.2 ± 19.8	333.0 ± 33.6	332.5 ± 23.5	0.318
CE2 (%)	31.8 ± 4.3	31.4 ± 8.0	35.7 ± 4.5	41.5 ± 8.1	47.3 ± 6.3	0.377

Data is represented as mean ± S.E.M., CA = clavulanic acid, EF= ejaculation frequency, LM1 = latency to 1st mount, LI1 = latency to 1st intromission, MF1 = mount frequency in the 1st ejaculatory series, IF1 = intromission frequency in the 1st ejaculatory series, LE1 = latency to the 1st ejaculation, PEL1 = 1st post-ejaculatory latency, CE1 = 1st series copulatory efficiency, MF2 = mount frequency in the 2nd ejaculatory series, IF2 = intromission frequency in the 2nd ejaculatory series, LE2 = latency to the 2nd ejaculation, PEL2 = 2nd post-ejaculatory latency, CE2 = 2nd series copulatory efficiency.

[a] $P<0.05$ significantly different than vehicle.

Table 2
Effects of chronic administration of clavulanic acid and paroxetine on sexual behaviour in male Wistar rats — sub-chronic treatment Day 7.

Parameter	Vehicle	Paroxetine	CA 0.01 mg/kg	CA 0.1 mg/kg	CA 1 mg/kg	ANOVA sig.
EF (#)	1.9 ± 0.3	0.9 ± 0.3	3.0[a] ± 0.2	3.4[a] ± 0.2	3.3[a] ± 0.2	0.000
LM1 (s)	28.3 ± 9.7	168.5 ± 148.5	17.8 ± 5.9	41.9 ± 24.9	89.0 ± 51.2	0.560
LI1(s)	34.0 ± 14.5	130.0[a] ± 47.8	22.5 ± 5.2	11.7 ± 2.4	32.6 ± 8.6	0.004
MF1 (#)	22.0 ± 4.0	41.7 ± 10.0	12.3 ± 3.8	6.0 ± 1.2	8.7 ± 2.5	0.000
IF1 (#)	9.9 ± 0.8	10.0 ± 1.7	7.7 ± 1.0	7.1 ± 0.6	6.0[a] ± 0.6	0.031
LE1 (s)	761.9 ± 148.1	1101.7 ± 202.1	371.7 ± 94.6	263.6 ± 40.5	314.3 ± 70.6	0.000
PEL1 (s)	265.4 ± 19.1	1035.3 ± 231.3	258.8 ± 20.3	283.2 ± 14.8	240.2 ± 28.9	0.000
CE1 (%)	37.4 ± 5.8	34.4 ± 8.5	45.9 ± 5.5	59.2 ± 4.8	52.6 ± 7.3	0.051
MF2 (#)	15.8 ± 4.8	ND	10.3 ± 2.8	6.1 ± 1.6	8.9 ± 3.0	0.300
IF2 (#)	3.9 ± 0.5	ND	3.9 ± 0.4	3.9 ± 0.3	3.7 ± 0.6	0.902
LE2 (s)	241.4 ± 55.5	ND	245.6 ± 53.1	189.5 ± 37.9	248.1 ± 58.9	0.798
PEL2 (s)	404.7 ± 28.4	ND	351.4 ± 28.9	339.6 ± 25.7	364.9 ± 15.3	0.916
CE2 (%)	30.6 ± 7.7	ND	34.7 ± 5.4	48.0 ± 6.2	43.9 ± 8.2	0.440

Data is represented as mean ± S.E.M., CA = clavulanic acid, EF= ejaculation frequency, LM1 = latency to 1st mount, LI1 = latency to 1st intromission, MF1 = mount frequency in the 1st ejaculatory series, IF1 = intromission frequency in the 1st ejaculatory series, LE1 = latency to the 1st ejaculation, PEL1 = 1st post-ejaculatory latency, CE1 = 1st series copulatory efficiency, MF2 = mount frequency in the 2nd ejaculatory series, IF2 = intromission frequency in the 2nd ejaculatory series, LE2 = latency to the 2nd ejaculation, PEL2 = 2nd post-ejaculatory latency, CE2 = 2nd series copulatory efficiency, ND = data not determined since $n<7$.
[a] $P<0.05$ significantly different than vehicle.

treatment test) and animals did not receive injections before this test. Experimental groups were randomly divided over all treatment groups and over the experimental days.

The behavioural experiments per testing day (acute, sub-chronic (Day 7), chronic (Day 14) and post-treatment (Day 21)) were performed over two successive days per week. Testing was performed between 9:00AM and 3:00PM in the dark phase of the LD-cycle under dim red light conditions. Males were injected with the assigned treatment and 30 min later placed into the experimental cage. After 30 min an oestrus female was placed into the cage and the behaviour of the male was scored over the ensuing 30-min. The female was checked for receptive and proceptive behaviour before the actual tests started.

The following parameters were scored using the Noldus Observer 5.0 program: mounts (frequency per ejaculation series and time of first mount in series, intromissions (frequency per ejaculation series and time of first intromission in series), number of ejaculations per test of 30 min, time of occurrence of ejaculations. From these data the following parameters were deduced: number of ejaculations/test; latency to 1st mount (s) in the 1st ejaculation series; latency 1st intromission (s) in the 1st ejaculation series; number of mounts in the 1st ejaculation series; number of intromissions in the 1st ejaculation series; latency to the 1st ejaculation (s) — time from the first mount or intromission to the first ejaculation; post-ejaculatory latency (s) — time from the 1st ejaculation till the first mount or intromission (whichever comes first) from the second ejaculatory series and the copulatory efficiency that is calculated as (# intromissions / (# intromissions + # mounts)) * 100%. All parameters are measured again for the second ejaculation series.

2.1 Missing values

The maximum value of 1800 was placed for latency to 1st intromission, 1st mount, 1st ejaculation, and 1st post-ejaculatory latency for any animal that failed to display those behaviours. Behavioural parameters in treatment groups with less than 7 data points ($n<7$) are given in the tables but statistics is not reliable because of the low number of contributing animals.

2.2 Statistics

Previous experiments show that the data obtained from sexual behaviour experiments are normally distributed. All data are analyzed using ANOVA using SPSSv11.0, followed by Bonferroni–post-hoc tests in case of overall significant effects.

The stability of training ejaculation frequencies were assessed with the non-parametric Kruskal Wallis test. All experiments were approved by the Ethical committee of Utrecht University (DEC GNK/FSB).

Table 3
Effects of chronic administration of clavulanic acid and paroxetine on sexual behaviour in male Wistar rats — chronic treatment Day 14.

Parameter	Vehicle	Paroxetine	CA 0.01 mg/kg	CA 0.1 mg/kg	CA 1 mg/kg	ANOVA sig.
EF (#)	2.3 ± 0.3	0.9[a] ± 0.2	2.9 ± 0.2	3.4[a] ± 0.3	3.3[b] ± 0.2	0.000
LM1 (s)	77.1 ± 34.4	62.6 ± 27.8	13.5 ± 4.8	54.1 ± 18.3	19.6 ± 10.1	0.192
LI1(s)	56.9 ± 22.0	309.2 ± 151.7	21.5 ± 4.8	8.2 ± 1.3	29.6 ± 9.3	0.015
MF1 (#)	17.3 ± 4.3	30.8 ± 5.1	13.6 ± 3.9	8.4 ± 2.8	7.6 ± 2.0	0.000
IF1 (#)	11.6 ± 1.7	10.3 ± 1.8	7.8 ± 0.9	8.1 ± 0.5	6.8[b] ± 0.6	0.050
LE1 (s)	669.1 ± 104.3	1192.8[a] ± 146.8	444.8 ± 102.3	265.8[a] ± 37.1	292.7[b] ± 42.8	0.000
PEL1 (s)	306.0 ± 26.9	1084.8[a] ± 216.3	259.7 ± 23.6	253.7 ± 13.0	271.9 ± 13.3	0.000
CE1 (%)	47.7 ± 6.1	29.4 ± 6.1	46.5 ± 6.1	60.2 ± 6.3	55.2 ± 7.0	0.014
MF2 (#)	11.2 ± 3.3	ND	8.8 ± 1.9	7.6 ± 1.4	3.7 ± 0.9	0.113
IF2 (#)	4.3 ± 0.4	ND	3.7 ± 0.5	3.8 ± 0.2	3.5 ± 0.2	0.145
LE2 (s)	278.2 ± 51.1	ND	244.7 ± 41.6	179.3 ± 20.2	133.9[a] ± 12.9	0.010
PEL2 (s)	336.4 ± 17.4	ND	342.5 ± 20.5	336.9 ± 15.3	310.1 ± 20.7	0.053
CE2 (%)	39.5 ± 8.6	ND	36.7 ± 6.4	39.3 ± 5.6	55.1 ± 6.3	0.307

Data is represented as mean ± S.E.M., CA = clavulanic acid, EF= ejaculation frequency, LM1 = latency to 1st mount, LI1 = latency to 1st intromission, MF1 = mount frequency in the 1st ejaculatory series, IF1 = intromission frequency in the 1st ejaculatory series, LE1 = latency to the 1st ejaculation, PEL1 = 1st post-ejaculatory latency, CE1 = 1st series copulatory efficiency, MF2 = mount frequency in the 2nd ejaculatory series, IF2 = intromission frequency in the 2nd ejaculatory series, LE2 = latency to the 2nd ejaculation, PEL2 = 2nd post-ejaculatory latency, CE2 = 2nd series copulatory efficiency, ND = data not determined since $n<7$.
[a] $P<0.05$ significantly different than vehicle.
[b] Marginally different than vehicle $0.05<P<0.10$.

3. Results

The average number of ejaculation for the 60 animals used in these 5 groups was 2.3 ± 0.1 on the last training day (day −7 in Fig. 1). Over the 5 experimental days the number of ejaculations remained unchanged ($\chi^2 = 1.704$, $P = 0.790$) indicating the very stable character of the sexual behaviour of the rats.

3.1. Day 1: Acute administration

After acute treatment all groups displayed on average between 1.6 and 3.0 ejaculations/30-min. tests (Fig. 1). Clavulanic acid had limited effects on sexual behaviour after acute dosing; only after the 1.0 mg/kg dose of clavulanic acid one significant effect was observed, indicating a weak pro-sexual effect: the number of intromissions in the first ejaculation series was decreased. Paroxetine had some mild inhibitory effects: it increased the latency to the 1st intromission and decreased the number of intromissions in the 1st series (Table 1).

3.2. Day 7: Sub-chronic administration

After 7-days treatment, clavulanic acid clearly showed pro-sexual activities at all doses used. The number of ejaculations was considerably enhanced (Fig. 1), although no dose-dependent effect was seen. Concomitant, some other changes were seen supporting the pro-sexual activity of clavulanic acid: decreases in the number of intromissions in the 1st ejaculation series (Fig. 2). Paroxetine clearly reduced sexual behaviour, although not strongly significant in all parameters. Its effect can be most clearly seen in the absence of the completion of the 2nd ejaculation series. Because only one series was completed (and not even in all animals) many parameters did not generate enough data points to make statistical evaluation worthwhile (Table 2).

3.3. Day 14: Chronic administration

After 14-days of chronic treatment, clavulanic acid induced a similar behavioural profile (pro-sexual) as after the 7 days sub-chronic treatment. Although the lowest dose tested showed no significant effects, strong statistical tendencies were present. Additionally, at the higher doses tested, clavulanic acid showed reductions in the latency to the 1st ejaculation (Fig. 2) and reduction in the number of intromissions in the 1st series paroxetine still exerted its strong inhibitory profile: a decrease in the number of ejaculations and strong increases in the latencies to the 1st (Fig. 2) and 2nd ejaculation and the 1st post-ejaculatory latency (Table 3).

3.4. Day 21: One week post-treatment

One week after cessation of treatment all effects of clavulanic acid have disappeared except for the latency to the 2nd ejaculation at the highest dose. The paroxetine treated group also completely returned to normal (Table 4).

4. Discussion

According to Rexahn, preliminary studies in monkeys had indicated that clavulanic acid potentially exerted some pro-sexual activities. To this end we performed a sexual behaviour study in male rats that were selected on basis of their sexual performance in a number of training tests. We selected animals with normal sexual behaviour (around 2 ejaculations per 30-min) which enables the detection of putative sexual stimulating (pro-sexual) and inhibiting effects. As a reference compound one dose of the inhibitory SSRI antidepressant paroxetine was included. Paroxetine indeed inhibited sexual behaviour and did so clearly after (sub) chronic administration in line with our earlier observations (De Jong et al., 2005). Clavulanic acid has a remarkable sexual stimulating (pro-sexual) activity. This profile is not yet fully present after acute treatment, although some indications of it are already seen (decreased latency to ejaculate), but becomes very evident after 7 and 14 days treatment, and also at all doses (0.01–1 mg/kg), although the effect of the 0.01 mg/kg dose seems to weaken a bit over time. The most intriguing finding is the increased number of ejaculations reached by the clavulanic acid-treated animals. After sub-chronic dosing of 7 and 14 days we see an increase of over 60% in this parameter. This seems primarily due to decreased latencies to the first (and to a lesser extent to 2nd) ejaculations. Because of these shortened latencies the animals seem to be more efficient although the copulatory efficiencies are not significantly improved, but the number of mounts and intromission are clearly diminished. More detailed studies are clearly needed to discern whether clavulanic acid exerts its pro-sexual activities via motivational or sexual execution mechanisms or both. As the cerebrospinal fluid/plasma ratio is 0.25, clavulanic acid apparently readily passes the blood-brain barrier. It might alter brain chemistry in some way to achieve pro-sexual activity. Preliminary in vivo microdialysis studies in rats following clavulanic acid treatment (unpublished data) revealed a time-dependent enhanced neurotransmission of serotonin and dopamine in the nucleus accumbens. The neurotransmitters serotonin and dopamine coordinate sexual motivation, copulatory behaviour and erectile function (Hull et al., 2004; Giuliano and Allard, 2001). Further studies are ongoing to elucidate its mechanism of action in the brain.

Table 4
Effects of chronic administration of clavulanic acid and paroxetine on sexual behaviour in male Wistar rats — extinction treatment Day 21.

Parameter	Vehicle	Paroxetine	CA 0.01 mg/kg	CA 0.1 mg/kg	CA 1mg/kg	ANOVA sig.
EF (#)	2.0 ± 0.3	2.3 ± 0.3	2.8 ± 0.4	3.0 ± 0.3	3.2 ± 0.4	0.076
LM1 (s)	19.4 ± 9.0	34.6 ± 9.4	72.1 ± 51.7	23.0 ± 10.3	62.5 ± 18.8	0.498
LI1(s)	38.5 ± 11.4	228.6 ± 148.0	19.5 ± 4.5	21.0 ± 11.1	28.4 ± 16.6	0.137
MF1 (#)	19.2 ± 3.2	12.3 ± 2.2	11.8 ± 4.3	13.7 ± 4.0	7.6 ± 2.3	0.189
IF1 (#)	9.6 ± 1.0	9.8 ± 1.3	7.2 ± 0.9	9.8 ± 1.8	6.5 ± 0.7	0.147
LE1 (s)	660.8 ± 101.8	633.0 ± 140.5	308.0 ± 58.7	464.0 ± 128.0	352.3 ± 70.0	0.424
PEL1 (s)	272.7 ± 15.2	260.1 ± 17.4	265.1 ± 15.5	271.6 ± 7.6	265.9 ± 19.2	0.727
CE1 (%)	37.5 ± 5.0	46.6 ± 5.7	52.5 ± 6.9	49.5 ± 4.7	56.7 ± 7.1	0.225
MF2 (#)	11.7 ± 3.0	8.1 ± 2.0	7.3 ± 2.3	8.0 ± 2.2	6.7 ± 2.7	0.671
IF2 (#)	4.4 ± 0.6	4.9 ± 0.7	2.7 ± 0.4	3.9 ± 0.5	3.8 ± 0.2	0.030
LE2 (s)	269.4 ± 30.2	245.2 ± 31.1	188.7 ± 27.8	188.9 ± 27.2	134.0[a] ± 18.0	0.010
PEL2 (s)	371.6 ± 13.0	394.1 ± 18.7	362.3 ± 25.3	329.2 ± 33.4	327.6 ± 22.2	0.274
CE2 (%)	36.4 ± 8.1	43.0 ± 6.9	36.8 ± 6.2	41.0 ± 5.1	55.6 ± 7.9	0.271

Data is represented as mean ± S.E.M., CA = clavulanic acid, EF= ejaculation frequency, LM1 = latency to 1st mount, LI1 = latency to 1st intromission, MF1 = mount frequency in the 1st ejaculatory series, IF1 = intromission frequency in the 1st ejaculatory series, LE1 = latency to the 1st ejaculation, PEL1 = 1st post-ejaculatory latency, CE1 = 1st series copulatory efficiency, MF2 = mount frequency in the 2nd ejaculatory series, IF2 = intromission frequency in the 2nd ejaculatory series, LE2 = latency to the 2nd ejaculation, PEL2 = 2nd post-ejaculatory latency, CE2 = 2nd series copulatory efficiency.
[a]$P<0.05$ significantly different than vehicle.

Our studies document a new property of clavulanic acid demonstrating that clavulanic acid exerts some putative pro-sexual effects. This is, to our knowledge, the first evidence of stimulatory enhancement of sexual activity by clavulanic acid in male rats. Further research is needed to investigate the mechanisms underlying this exciting finding.

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