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Effects of salvinorin A on locomotor sensitization to D2/D3 dopamine agonist quinpirole

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ABSTRACT

Locomotor sensitization induced by the dopamine agonist quinpirole can be potentiated by co-treatment with the synthetic kappa opioid agonist U69593. The identification of salvinorin A, an active component of the psychotropic sage *Salvia divinorum*, as a structurally different agonist of kappa-opioid receptors raised the question of whether this compound would similarly potentiate sensitization to quinpirole. Rats were co-treated with 0.5 mg/kg quinpirole and either salvinorin A (0.04, 0.4 or 2.0 mg/kg) or U69593 (0.3 mg/kg). Control groups were co-treated with vehicle and saline, vehicle and quinpirole (0.5 mg/kg), or saline and salvinorin A (0.4 mg/kg). Rats were injected biweekly for a total of 10 injections and locomotor activity measured after each treatment. Results showed that the highest dose of salvinorin A potentiated sensitization to quinpirole as did U69593, the middle salvinorin A dose had no effect on quinpirole sensitization, and the lowest dose of salvinorin A attenuated sensitization to quinpirole. These findings indicate that structural differences between salvinorin A and U69593 do not affect the potentiation of quinpirole sensitization. Moreover, the opposite effects of high and low salvinorin A doses suggest that salvinorin A can produce bidirectional modulation of sensitization to dopamine agonists.

There is growing interest from both the general public and the scientific community in the psychotropic sage, *Salvia divinorum* (Lamiaceae). Originally cultivated in southern Mexico for medicinal and religious purposes amongst the Mazatec people [16], preparations of this plant have become widely available in North America. Ingestion of leaves of the plant can modify conscious experience and induce distinct visual and cognitive effects [3]. The plant currently enjoys increasing recreational use [1,4], probably due to the lack of restrictive legal controls on its use and supply.

The principle active ingredient of *S. divinorum* was isolated in 1982 [6] and named salvinorin A [14]. The recent identification of salvinorin A as a highly selective and potent ligand for kappa opioid receptors (κOR) [10] was surprising because the subjective effects of salvinorin A were thought to be similar to those of serotonin receptor agonists, such as psilocybin, which is found in *Psilocybe cubensis* mushrooms. Subsequent studies revealed the rather unique structural properties of this kappa agonist. Specifically, unlike other known κOR agonists, salvinorin A does not contain an ionizable amine group, a moiety that was thought to be essential for interaction with the κOR [5]. Instead, non-polar interactions seem to be critical for the binding of salvinorin A to κOR, as proposed in a new model [5]. This kind of interaction results in a different binding configuration when compared to κOR agonists possessing the amine moiety. Conceivably, these differences in binding configuration have distinct functional consequences. Indeed, differences between the effects of salvinorin A and the kappa agonist U695593 on behavior have been observed in one study [15].

We have recently reported that U69593 has a marked effect on behavioral sensitization to quinpirole, a dopamine D2/D3 agonist. Specifically, the co-administration of U69593 and quinpirole accelerates the development of quinpirole-induced locomotor sensitization [7], quinpirole-induced compulsive checking [9] as well as increasing the magnitude of the sensitized locomotor response [7,9]. Considering the difference between U69593 and salvinorin A in terms of interaction at the binding site, we asked in the present study whether the potentiating effects of U69593 on sensitization to quinpirole were particular to kappa agonists with the ionizable amine moiety or extend to structurally different agonists such as salvinorin A. Our results showed that the co-administration of salvinorin A and quinpirole had effects comparable to co-treatment of

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U69593 and quinpirole. However, when a full dose–response profile for salvinorin A was evaluated, an unexpected attenuation to quinpirole sensitization was observed at the lowest dose of salvinorin A (0.04 mg/kg).

Eighty naïve, male Long-Evans rats (Charles River, Canada) weighing 250–300 g at the start of the experiment were housed individually in polyethylene cages (35 cm × 30 cm × 16 cm), lined with Tek-Fresh Laboratory bedding made from virgin wood pulp (Harlan Teklad, Madison, WI). The cages were located in a temperature controlled (22 °C) colony room on a 12 h light–dark cycle (lights on at 07:00) and with food and water freely available. Upon arrival from the supplier, the animals were allowed to acclimatize to the colony room for a week followed by a subsequent week of handling by the experimenter. Specifically, during the first 2 days, individual rats were removed from their home cage by each of the four experimenters and placed on the experimenter's arm for about 2 min. On day 3, rats were weighed in a Dial-O-Gram balance (Ohaus Scale Corporation, Florham Park, NJ). During the next 2 days, rats underwent the same holding procedure as for the injection of drugs, except that no injection or needle prick was administered. From day 3 onwards, individual rats were handled by the same experimenter throughout the study. All tests were performed during the light phase of the day–night cycle. Procedures and housing were in accordance with the guidelines described in the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

All chemicals and reagents other than salvinorin A were purchased from Sigma–Aldrich (Oakville, Ontario). Salvinorin A (>98% purity) was provided by Dr. D. Siebert (The *Salvia divinorum* Research and Information Center, Malibu, CA). Quinpirole hydrochloride (QNP; 0.5 mg/kg) was dissolved in physiological saline, while salvinorin A (0.04; 0.4; and 2.0 mg/kg) and U69593 (0.3 mg/kg) were dissolved in a vehicle comprised of dimethyl sulfoxide (DMSO) and propylene glycol (1:1). When two drugs were co-administered, the κOR agonist U69593 or salvinorin A was administered first, immediately followed by the quinpirole injection. For non-drug injections an equivalent volume of vehicle or physiological saline was substituted for κOR agonists and quinpirole respectively. All drugs were injected at a volume of 1 ml/kg.

The VersaMax Animal Activity Monitoring System (AccuScan Instruments, Columbus, OH) was used to measure locomotor activity automatically in eight Plexiglas activity chambers (40 cm × 40 cm × 35 cm). The system consisted of a Digiscan 16 analyzer that monitored the state of 30 infra-red beams forming a horizontal *X*–*Y* grid over the bottom of the activity chamber, and a computer interface with VersaMax software that captured the beam breaks and derived from their sequence and timing a measure of distance travelled. Activity chambers were located in an experimental room that was separate from, but adjacent to the colony room. The cages were covered with ventilated Plexiglas lids to prevent animals from jumping out.

To examine the effects of salvinorin A on the development of locomotor sensitization to quinpirole, a design similar to one used previously was employed [7]. Rats were randomly assigned to one of seven groups that were balanced by weight. Three groups were co-treated with quinpirole (0.5 mg/kg) and salvinorin A (0.04, 0.4 or 2.0 mg/kg; $n = 12$/dose). Control groups were co-treated with vehicle and saline ($n = 10$), vehicle and quinpirole (0.5 mg/kg; $n = 11$), or saline and salvinorin A (0.4 mg/kg; $n = 11$). A seventh group was co-treated with quinpirole (0.5 mg/kg) and U69593 (0.3 mg/kg; $n = 12$), and served as a positive control group to verify the presence in this study of the expected potentiation of quinpirole sensitization with a κOR agonist [7]. The doses of salvinorin A were selected such that the highest dose (2 mg/kg) corresponded to the dose commonly used in behavioral studies in rats (e.g., [2]), and the lowest dose (0.04 mg/kg) approximated that of human recreational use [11]; the 0.4 mg/kg dose of salvinorin A was chosen as an intermediate dose within the selected dose range. The comparison dose of U69593 (0.3 mg/kg) was the same as the dose previously found to markedly enhance locomotor sensitization [7]. Animals were injected biweekly for a total of 10 injections, a protocol that induces robust sensitization to quinpirole alone [7].

Prior to each test, animals were weighed and transported in their home cages to the experimental room. To administer the drugs, rats were taken out of the home cage, placed on a towel resting flat on a cart, and injected subcutaneously under the nape of the neck. Immediately after the injections, animals were individually placed in the activity chambers for 60 min and locomotor activity was measured. Once the testing period had elapsed, activity chambers were thoroughly cleaned with Windex diluted in water.

For each treatment group, the mean of distance traveled at each injection was computed and the parameters providing the best fit for the following asymmetric sigmoid equation were estimated using a nonlinear curve-fitting algorithm (Fig.P Version 2.98, Fig.P Software Corporation, Hamilton, Ontario, Canada).

$$R = \frac{R_{\min} + (R_{\max} - R_{\min})}{1 + (X/X_{50})^{-n}}$$

where X is the number of injections (I) and R is the response after I number of quinpirole injections. The estimated parameters are: $R_{\max}$, the maximal response after an infinite number of injections; X_{50} (I_{50}), the number of injections to reach the half-maximum response; and, n, some power coefficient that represents the sigmoidicity of the function. In computing R, $R_{\min}$ was a fixed parameter and was set equal to the mean value of distance travelled at injection 1. The use of this function for sensitization to quinpirole was described previously [12,13]. The parameters, I_{50} and $R_{\max}$ were taken as measures of the speed of sensitization and of the maximum magnitude of sensitization attained, respectively. To evaluate the effects of chronic treatments on the sensitization to quinpirole, the best-fit parameters were compared between treatment groups using two-tailed t-tests. Data were expressed as mean ± standard error of mean.

As expected, repeated injections of quinpirole-induced locomotor sensitization, as evidenced by a fourfold increase in the distance traveled compared to the acute drug response (Fig. 1). Moreover, as found previously [7], the co-administration of U69593 and quinpirole potentiated locomotor sensitization to quinpirole (Fig. 1), elevating the maximal response ($R_{\max}$) and reducing the number of injections needed to reach the half-maximal response (I_{50}) (Table 1). The effect of co-administered salvinorin A was dose dependent. The highest dose (2 mg/kg) produced a locomotor sensitization profile that was indistinguishable from U69593 and significantly different from injections of quinpirole-alone (Fig. 1 and Table 1). The middle dose (0.4 mg/kg) had no apparent effects on sensitization to quinpirole (Fig. 1). Unexpectedly, co-administration of the lowest dose of salvinorin A (0.04 mg/kg) attenuated quinpirole sensitization (Fig. 1), producing a statistically significant reduction in $R_{\max}$ compared to the highest and middle doses of salvinorin A, and a trend for elevating the I_{50} (Table 1).

The locomotor response to injections of salvinorin A-alone was not different from that of saline-injected controls (Fig. 1).

The present findings show that, at high doses, the co-administration of salvinorin A and quinpirole had effects comparable to co-treatment with U69593 and quinpirole. The high dose of salvinorin A (2 mg/kg) co-administered with quinpirole resulted in a similar potentiation of locomotor sensitization to quinpirole as observed with U69593. Thus, both κOR agonists, with or without the ionizable amine moiety, can potentiate quinpirole

Table 1
Estimates ±S.E. of parameters of a sigmoid function fitted to data presented in Fig. 1

Variable	Group	Parameter[a]				r^2
		I_{50}	R_{max}	n	R_{min}	
Average distance (m/min)	VehQ	3.08 ± 0.15	7.12 ± 0.21	3.17 ± 0.46	1.56	0.987
	U69Q	2.48 ± 0.23**	8.48 ± 0.35**	2.54 ± 0.60	2.70	0.963
	SVLQ	3.70 ± 0.38	6.25 ± 0.44*	2.49 ± 0.52	1.18	0.976
	SVMQ	3.16 ± 0.18	7.50 ± 0.28a	2.73 ± 0.40	1.32	0.987
	SVHQ	2.62 ± 0.20*a,c	8.58 ± 0.29**a,b	2.66 ± 0.53	2.84	0.975

[a] Equation fitted to the data of the indicated groups shown in Fig. 1. I_{50} is the number of drug injections required to reach the half-maximal response, R_{max} is the maximal response, n is a parameter describing the sigmoidicity of the curve, R_{min} is the lowest response that served as a fixed parameter in the equation, and r^2 indicates the square of the correlation coefficient between raw and fitted data. Standard error (S.E.) refers to the standard error of the estimate of the parameter; the estimate of each parameter is statistically significant. Group abbreviations correspond to those in the figure: VehQ, vehicle plus quinpirole; U69Q, U69593 plus quinpirole; SVLQ, salvinorin A (0.04 mg/kg) plus quinpirole; SVMQ, salvinorin A (0.4 mg/kg) plus quinpirole; SVHQ, salvinorin A (2 mg/kg) plus quinpirole. a, $p \leq .05$ compared to SVLQ, *t*-test, two-tailed; b, $p \leq 05$ compared to SVMQ, *t*-test, two-tailed; c, $p \leq .10$ compared to SVMQ, *t*-test, two-tailed.

* $p \leq .10$ compared to VehQ group, *t*-test, two-tailed.

** $p \leq .05$ compared to VehQ group, *t*-test, two-tailed.

sensitization. Therefore, it appears that the potentiating effect of co-activation of κOR and D2/D3 dopamine receptors on quinpirole sensitization is not influenced by the structural differences between the two kappa opioid agonists.

Despite the ability of both kappa opioid agonists to potentiate locomotor sensitization, an unexpected attenuation of quinpirole sensitization was observed when the lowest dose of salvinorin A (0.04 mg/kg) was co-administered (the intermediate dose of salvinorin A had no effect on quinpirole sensitization). The attenuation was unexpected because in our previous co-treatment studies only potentiation had been demonstrated [7–9], although the dose range of U69593 that had been explored was relatively narrow (0.15 and 0.3 mg/kg). Thus, the results of the present study are insufficient to establish whether the bidirectional modulation of locomotor sensitization is particular to salvinorin A or applies to κOR agonists in general. As discussed previously [7], κOR agonists may influence sensitization and dopamine neurotransmission through both pre- and postsynaptic mechanisms that have differing sensitivities to the agonist and which may exert opposite effects on dopamine signal transduction. It is conceivable, therefore, that the dose of salvinorin A can determine attenuation or enhancement of locomotor sensitization, depending on the extent to which these separate mechanisms are activated.

In summary, the co-administration of salvinorin A (2 mg/kg), like that of U69593, can potentiate locomotor sensitization to quinpirole, suggesting that such an effect does not necessitate the presence of an ionizable amine group in the agonist. Yet, the co-administration of a low dose of salvinorin A (0.04 mg/kg) has an opposite effect, attenuating quinpirole sensitization. Further studies are necessary to establish whether such bidirectional effects are particular to salvinorin A or are a general feature of κOR agonists. Regardless, the present findings point to the potential utility of different κOR agonists in potentiating and attenuating the activity of dopamine signaling.

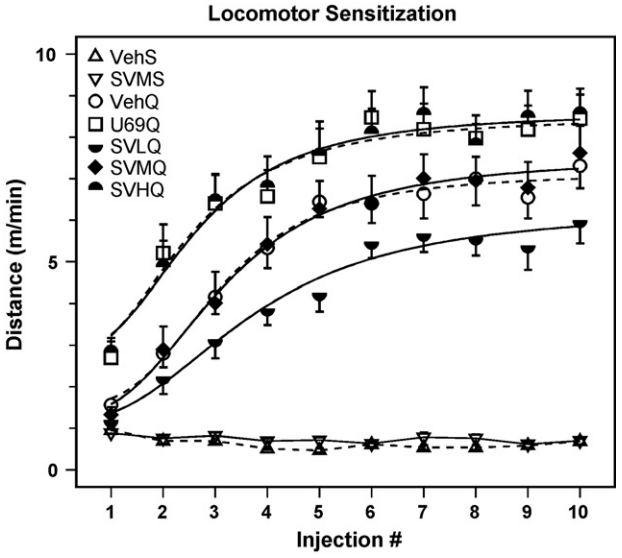

Fig. 1. Development of locomotor sensitization to repeated injections of quinpirole and its modulation by co-treatment with U69593 or salvinorin A. Locomotor activity is measured as total distance traveled during the 60 min test session and is normalized to the duration of the test session in units of m/min. Smooth lines show the best fit estimate of the asymmetric sigmoid equation indicated in the text section, with parameters given in Table 1; straight lines connecting group symbols indicate that the function did not fit those data, consistent with no evidence of locomotor sensitization. Injections were administered 3–4 days apart. Legend: VehS, vehicle plus saline; SVMS, salvinorin A (0.4 mg/kg) plus saline; VehQ, vehicle plus quinpirole (0.5 mg/kg); U69Q, U69593 (0.3 mg/kg) plus quinpirole (0.5 mg/kg); SVLQ, salvinorin A (0.04 mg/kg) plus quinpirole (0.5 mg/kg); SVMQ, salvinorin A (0.4 mg/kg) plus quinpirole (0.5 mg/kg); SVHQ, salvinorin A (2 mg/kg) plus quinpirole (0.5 mg/kg).

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