Pharmacology Biochemistry & Behavior, Vol. 16, pp. 249–252, 1982. Printed in the U.S.A.

D-Ala^2,D-Leu^5-Enkephalin Generalizes to a Discriminative Stimulus Produced by Fentanyl but not Ethylketocyclazocine

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Received 29 July 1981

SHEARMAN, G. T. AND A. HERZ. *D-Ala^2,D-Leu^5-enkephalin generalizes to a discriminative stimulus produced by fentanyl but not ethylketocyclazocine.* PHARMAC. BIOCHEM. BEHAV. 16(2) 249–252, 1982.—Male Sprague Dawley rats were trained to discriminate between the effects of saline and either fentanyl (0.04 mg/kg) or ethylketocyclazocine (0.32 mg/kg) by responding on a FR 10 schedule of food reinforcement with a lever on one side of a food cup following a subcutaneous saline injection and responding with a lever on the alternate side following the subcutaneous injection of one of those drugs. The effects of an intracerebroventricular injection of either fentanyl (2.89–14.45 nmol) or D-Ala^2,D-Leu^5-enkephalin (0.172–1.72 nmol) dose-dependently generalized to the discriminative stimulus produced by fentanyl injected subcutaneously. Intracerebroventricular injection of ethylketocyclazocine (15.7–125.8 nmol) but not D-Ala^2,D-Leu^5-enkephalin (1.72–13.76 nmol) dose-dependently generalized to the discriminative stimulus produced by ethylketocyclazocine injected subcutaneously. These data demonstrate similarities in the discriminative stimulus properties of proposed μ and δ but not κ and δ opiate receptor agonists.

Narcotic discrimination Fentanyl Ethylketocyclazocine D-Ala^2,D-Leu^5-enkephalin Naloxone Multiple opiate receptors

IT is well established that narcotic drugs can function as discriminative stimuli to reliably control the operant behavior of laboratory animals (for reviews see [6, 10, 12]). Recently, it was suggested that differences in the discriminative stimulus properties amongst narcotics are related to their interaction with different opiate receptors [9, 19, 23].

It was the purpose of this study to evaluate the discriminative stimulus effects of the proposed opiate δ receptor agonist [14, 21, 29–32] D-Ala^2,D-Leu^5-enkephalin (DADL) in rats trained to discriminate between the effects of saline and either the proposed opiate μ receptor agonist [16, 21, 30–32] fentanyl or the proposed κ receptor agonist [11, 15, 21] ethylketocyclazocine (EKC). We now report that DADL generalizes to a discriminative stimulus produced by fentanyl but not EKC.

METHOD

Animals

Male Sprague Dawley rats (Musratus GmbH., Brunnthal, F.R.G.) weighing between 300 and 350 g were used. The animals were housed in single cages in a large colony room thermostatically maintained at 24±1°C. The room lights were turned on from 7:00 a.m. to 7:00 p.m. Water was continuously available in the home cages but food was restricted to 20 g a day made available approximately 4 hr following each operant session.

Apparatus

The behavioral apparatus consisted of six identical conventional Skinner boxes housed in lightproof, sound-attenuated, and fan-ventilated chambers. Each Skinner box contained a houselight which was located above a food receptacle that was installed in the center of one wall equidistant from two response levers. Scheduling of behavioral contingencies and recording of data was made with solid-state programming modules (Coulbourn Instrument Inc., Lehigh Valley, PA).

Discrimination Training

The rats were first trained to discriminate reliably between the effects of saline and either 0.04 mg/kg fentanyl (n=12) or 0.32 mg/kg EKC (n=12) according to the procedure of Colpaert *et al.* [7]. The rats were trained to respond with one of the levers 30 min following training drug injection and with the other lever 30 min following a saline (1 ml/kg) injection. Every tenth response (FR 10) with the appropriate lever resulted in the delivery of a food pellet. Responses with the inappropriate lever (i.e., drug lever after saline injection) were recorded but were not reinforced by the delivery of food.

The drug lever was on the right side of the food receptacle for half of the rats and on the left side for the remaining

animals. For each rat, the position of the drug and saline lever remained constant on each subsequent session. The sequence of drug-saline injections was varied separately for each group of rats trained successively on the same day. Initially, the sequence of drug-saline injections alternated. This training continued until five such alternations were achieved and responding was stabilized with the appropriate lever. Following this, the rats entered the final phase of training where drug-saline sessions were carried out 7 days a week according to an irregularly alternating sequence of drug-saline injections. In this and all subsequent phases of the experiment, the session length was fixed at 15 min. The rats were trained to a criterion of emitting four or less responses with the incorrect lever (i.e., drug lever after saline injection) prior to the first reinforcement (10 responses with the correct lever) on 9 out of 10 consecutive sessions.

Surgical Procedure

Following acquisition of this criterion level of performance, guide cannulas were implanted into the right lateral ventricle (for technical details see [13]). At the end of the experiment, 10 μl of 1% trypan blue solution was injected into the cannula and the rats were immediately sacrificed by decapitation to examine the placement of cannulas. Data were evaluated only from those animals in which the spread of dye indicated a correct injection site.

Discrimination Testing

One week following surgery, ten additional saline-fentanyl or saline-EKC practice sessions were given to insure maintenance of the discrimination. No deficit of retention of the discriminations was noticed. Discrimination tests consisted of 15 min sessions separated by at least four practice sessions in which four or less responses were emitted with the incorrect lever before lever selection. If the rat's performance on these practice sessions deteriorated with respect to the number of responses with the incorrect lever prior to lever selection, further training sessions were given before testing was reinstated. For half of the rats, test sessions were preceded by a saline practice session whereas for the remaining rats, test sessions were preceded by a drug practice session. For generalization testing, 15 min following intraventricular injection or 30 min following subcutaneous injection of the test drug each rat was placed in its assigned Skinner box and allowed to respond with levers. The lever with which 10 responses were emitted first was considered the selected lever and was subsequently fixed to be reinforced (FR 10) for the remainder of the test session. Responses emitted with the other lever were recorded but not reinforced.

Data Analyses

Data were expressed as a percentage of rats selecting training drug lever following each drug treatment. The Fisher Exact Probability Test was used to determine statistical significance of results. Regression analysis was performed to determine whether generalization to the training drug discriminative stimulus was a dose-related effect. ED 50's and 95% confidence limits were calculated according to the method of Finney [8].

TABLE 1

GENERALIZATION TESTS WITH RATS TRAINED TO DISCRIMINATE BETWEEN THE EFFECTS OF SUBCUTANEOUSLY ADMINISTERED FENTANYL (0.04 mg/kg) AND SALINE*

Test Drug	Dose (mg/kg)	N†	% Rats Selecting Fentanyl Lever
Fentanyl	0.04	12	100
Saline	—	12	0
	nmol/rat		
Fentanyl	2.89	6	0
	7.23	6	50
	14.45	6	100
Saline	—	6	0
DADL	0.172	6	0
	0.86	6	33
	1.72	6	100

*Following injection of the test drug, the rats were placed in their assigned Skinner boxes and allowed to make a lever selection. The lever with which ten responses were completed first was considered the selected lever.

†Number of rats tested.

Drugs

The following drugs were used: ethylketocyclazocine methanesulfonate (generously supplied by Sterling-Winthrop Research Institute, Rensselaer, NY), fentanyl citrate (Janssen Pharmaceutica, Beerse, Belgium), and D-Ala^2,D-Leu^5-enkephalin (Bachem Feinchemikalien AG, Bubendorf, Switzerland). Fentanyl and ethylketocyclazocine were dissolved in 0.9% sterile saline and administered SC in a volume of 1 ml/kg or intraventricularly in a volume of 5–10 μl. DADL was dissolved in 0.9% sterile saline and administered intraventricularly in a volume of 5 μl. Drug doses were calculated in terms of the free base. Doses of each test drug were administered in an irregular order.

RESULTS

As shown in Table 1, all of the rats selected the fentanyl lever after subcutaneous injection of fentanyl (0.04 mg/kg) whereas none selected this lever following a saline injection. Intraventricular injection of either fentanyl (2.89–14.45 nmol/rat; 1–5 μg/rat) or DADL (0.172–1.72 nmol/rat; 0.1–1.0 μg/rat) dose-dependently (r's=1.0 and 0.91 respectively, p's<0.05) generalized to the discriminative stimulus produced by fentanyl (0.04 mg/kg) administered subcutaneously. The ED 50's were 7.23 (5.58–9.34) and 1.01 (0.80–1.28) nmol/rat respectively.

Data summarized in Table 2 show that all of the rats injected subcutaneously with EKC (0.32 mg/kg) selected the EKC lever and none selected this lever after a saline injection. Whereas intraventricular injection of EKC (15.7–125.8 nmol/rat; 5–40 μg/rat) dose-dependently (r=0.99; p<0.05) generalized (ED 50 39.8 (28.8–55.0) nmol/rat) to the discriminative stimulus produced by EKC injected subcutaneously, DADL (1.72–13.76 nmol/rat; 1–8 μg/rat) did not.

TABLE 2

GENERALIZATION TESTS WITH RATS TRAINED TO DISCRIMINATE BETWEEN THE EFFECTS OF SUBCUTANEOUSLY ADMINISTERED ETHYLKETOCYCLAZOCINE (0.32 mg/kg) AND SALINE

Test Drug	Dose (mg/kg)	N	% Rats Selecting EKC Lever
EKC	0.32	12	100
Saline	—	12	0
	nmol/rat		
EKC	15.7	6	0
	31.4	6	33
	62.9	6	83
	125.8	6	100
Saline	—	6	0
DADL	1.72	6	0
	3.44	6	33*
	6.88	6	0
	13.76	3	No Selection†

For details see Table 1.

*$p<0.05$ versus EKC (0.32 mg/kg; SC or 125.8 nmol/rat; ICV), Fisher Exact Probability Test.

†No Selection indicates that ten responses were not emitted with either lever during the entire fifteen minute test session.

DISCUSSION

Recently, we reported [19] that fentanyl did not generalize to the discriminative stimulus produced by EKC and vice versa and that the discriminative stimulus properties of fentanyl and EKC were mediated by an interaction with μ and κ opiate receptors respectively. The present experiment demonstrates that the discriminative stimulus effects of the proposed δ opiate receptor agonist, DADL, dose-dependently generalize to the discriminative stimulus effects produced by fentanyl but not EKC. Previously, it was reported [29] that the effects of systemic administration of D-Ala^2,D-Leu^5-enkephalinamide (10 mg/kg) did not generalize to a discriminative stimulus produced by either the non-selective [21, 28–30] opiate agonist, etorphine or EKC. Although amidation of enkephalin decreases its selectivity for δ receptors [30–32], D-Ala^2-enkephalinamide [5] and D-Met^2-Pro^5-enkephalinamide [3] have been reported to generalize to a discriminative stimulus produced by the μ-agonist [15, 21, 28–30] morphine.

In agreement with our present finding are the reported similarities between μ and δ but not κ and δ opiate receptor agonists with respect to their effect on dopamine [26] and acetylcholine [25,27] metabolism. In addition, like fentanyl [20] but unlike κ agonists [28], the δ agonist [14, 30–32] leu-enkephalin was self-administered by laboratory animals [1,22].

The exact mechanism by which DADL generalized to the fentanyl discriminative stimulus remains to be determined. In drug binding studies, (^{3}H)-DADL displays at least a 500-fold higher affinity to δ than κ receptors but only a 5–8 fold higher affinity for δ than μ receptors [11,17]. Furthermore, 30% of (I^{125})-DADL has been shown to bind to μ receptors in rat brain [4]. Thus, the generalization of the effects of DADL to those of fentanyl but not EKC may be the result of its interaction with μ and δ but not κ opiate receptors. Alternatively, it was recently suggested that an interconversion [2] or coupling [18,24] occurs between μ and δ receptors. Therefore, interaction of DADL with the δ receptor may result in an interconversion or coupling to a μ receptor and result in a μ or fentanyl-like effect. The lack of DADL generalization to EKC in the present study suggests that interconversion or coupling between δ and κ receptors may not occur.

In summary, these data demonstrate similar discriminative stimulus effects of proposed μ and δ but not κ and δ opiate receptor agonists.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Bundesgesundheitsamt. The skilled technical assistance of Frau Ursula Bäuerle is greatly appreciated.

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