An Assessment of the Addiction Potential of the Opioid Associated with Milk

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ABSTRACT

Eighty-four male rats were tested to determine their preference for one of two distinctive places in an experimental space. After an initial determination of place preference, rats were assigned to six groups. They were then subjected to procedures to condition a place preference using doses of β-casomorphin, a standard dose of morphine, or placebo. Subsequently, rats were tested for place preferences. No evidence emerged indicating that injections of β-casomorphin conditioned a place preference, but evidence indicated that morphine conditioned a place preference. Consequently, systemically administered β-casomorphin has very limited or no reinforcing properties similar to those of morphine. Ingestion of milk products containing β-casomorphin is not likely to become the focus of an addiction.

(**Key words**: β-casomorphin, conditioned place preference, addiction potential, opioids)

Abbreviation key: **CPP** = conditioned place preference.

INTRODUCTION

Milk is a complex product containing a number of biologically active polypeptides (5) that could easily have "pharmacological" effects. Products associated with bovine milk have been discovered, for example, which have opioid (opiate) activity. Enzymatic digestion of β-casein of milk can produce peptides called β-casomorphins, which show activity similar to that of morphine in certain bioassays and bind to opioid receptors (2, 3, 4, 7, 8, 9, 10). These opioid peptides associated with food were called exorphins, in contrast to the endogenous opioids sometimes called endorphins.

Do these opioid products of milk produce "pleasurable" activity (positive affect or reward) or other events that are salient to addiction? This question has not been systematically addressed. How can an experimental subject, such as a rat, be asked whether or not the effects of a compound produce something akin to pleasure? Recently, considerable conceptual and technical advances have been made in development of techniques for answering the appropriate questions using rats, and a recent compendium of relevant information has been compiled (1). An efficient and widely used (6) procedure for gaining germane information is the conditioned place preference (**CPP**) test.

The CPP test is conceptually simple. Rats are assumed to prefer places where they experience positive affect, to avoid places where they experience aversiveness, and to be neutral toward places where no particular affective state is elicited. To translate those assumptions into a procedure, an alley was devised (11) having two distinct halves. Although the two sides of the alley were distinct, the arrangement was such that rats showed no marked preference for either side prior to conditioning. The two sides can be separated by a removable wall.

A standard CPP procedure exists using morphine as the agent to elicit positive affect in rats (11, 12). First, rats are habituated to the general procedures. Second, their initial preferences for one side of the alley are determined (a baseline measure) by measuring the time that each rat spends in a side of the alley during, for example, a 30-min period. Conditioning begins, and, finally, putative conditioning is tested.

Typically, conditioning sessions occur once daily for several days. On 1 d, a rat under the

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influence of morphine is placed in one side of the alley; the wall separating the sides is installed. On the next day, a rat under the influence of placebo is placed on the other side. This pairing of one side (place) with the effects of morphine and the other side with the effects of placebo continues for several days (often 6 d, 3 d with morphine and 3 d with placebo). Subsequent to the conditioning, a CPP test is conducted as the initial baseline test (i.e., a rat again had access to the entire alley, and the time spent in each side is tabulated).

After conditioning and at testing, rats show a preference for the place where they previously experienced the effects of an agent, or an event, known to produce positive affect among rats (as indexed by other tests). Rats prefer, for example, places where they experience the effects of certain doses of morphine, heroin, cocaine, and similar drugs [for a review, see (6)]. The general tendencies of rats to explore compete with their putative CPP; therefore, at testing, they rarely spend all of their time in the place of putative affective experience. Nevertheless, reliable preferences for a side are discernible by comparisons of rats given only placebos with rats given morphine.

Because a technology exists for determining whether β-casomorphin affects central neural events that are manifest as positive affect and because the ability of β-casomorphin to produce such effects is of interest, the following experiment was performed. The effects of intraperitoneal injections of large doses of β-casomorphin were assessed among rats using CPP procedures. Intraperitoneal injections, rather than oral infusions, were used to provide for potentially larger effective doses than might be otherwise achieved. The resulting doses are thought to be greater than any effective dose that might be achieved by ingestion of milk products.

MATERIALS AND METHODS

Rats

The subjects were 84 male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 175 to 200 g when purchased. Rats were individually housed in standard hanging cages in a vivarium maintained at 22°C with 12 h of light daily beginning at 1000 h. Food and water were always available in home cages.

Drugs

The doses of β-casomorphin acetate (bovine; AA sequence: Tyr-Pro-Phe-Pro-Gly-Pro-Ile; Sigma Chemical Co., St. Louis, MO) tested were 1.25, 2.5, 5.0, and 10.0 mg/kg. The vehicle for β-casomorphin was distilled water, which served as its placebo. The dose of morphine sulfate (Mallinckrodt Specialty Chemicals Co., St. Louis, MO) tested was 4.0 mg/kg. The vehicle for morphine was physiological saline, which served as its placebo. β-Casomorphin and its placebo were given intraperitoneally 2 min prior to conditioning sessions. Morphine and its placebo were given subcutaneously 15 min prior to conditioning sessions. All injections were given in volumes of 1.0 ml/kg.

Apparatus

The CPP apparatus, housed in a room adjacent to the vivarium, has been described in detail elsewhere (11). Briefly, the apparatus consisted of 12 nearly identical alleys, each housed in a sound-attenuating outer shell. Each side of any alley had distinct visual (solid grey or black and white striped sides) and textural cues (flooring made of steel rods running either parallel or perpendicular to the length of the alley). A barrier, with sides that match the respective halves of the alley, separated the two sides during conditioning.

The amount of reflected light on each side of an alley was adjusted so that the putative conditioning side was slightly brighter than the alternative side. Each alley was suspended on a center support so that it tilted slightly when a rat moved to one side. An electrical circuit closed when the box tilted, providing information, collated by a personal computer about the amount of time spent in each side of the alley.

Procedure

On the day following their arrival at the laboratory, the rats began a 3-wk schedule of habituation, conditioning, and testing. All procedures took place between 1100 and 1500 h.

Days 1 to 5 served as a handling phase in which the subjects were habituated to the general daily procedures. The rats were weighed, placed into a cart (12 cages per cart,

1 rat per cage), and transported into the room housing the apparatus, where each rat was handled briefly before being returned to its cage. On d 6 and 7, each rat was placed into its respective alley with access to both sides for 30 min. The time spent on the arbitrarily chosen, putative side of conditioning was recorded on d 7 and was the baseline measure. Subsequently, rats were divided into six groups (n = 12, except for the morphine controls for which n = 24) such that mean preferences of groups for the putative side of conditioning were nearly equal. The putative side of conditioning was usually the side with the slightly brighter lighting and, therefore, the side least preferred by the rats. A further restriction was that, for half of each group, the putative side of conditioning was the gray side and, for the other half, the striped side. Subsequently, treatments were randomly assigned to groups.

Across d 8 to 10, rats received their respective injections and were confined to the putative side of conditioning (Table 1). On d 11, all rats received placebo and were confined to the alternate side of conditioning (Table 1). Thus, across d 8 to 11, rats received their assigned injections before being confined to one side of the alley. All conditioning sessions lasted 20 min.

On d 12, a CPP test was given. The test session lasted for 30 min. Rats received no injections prior to the test session. Without interruption, this 5-d sequence of putative and alternate conditioning followed by a test for CPP was repeated again across d 13 to 17. Across d 18 to 21, 2 d were allotted to putative conditioning, 1 d to alternate conditioning, and a 3rd d to the CPP test.

TABLE 1. Injections received by rat groups prior to confinement to putative and alternate sides of conditioning.

Group	Putative	Dose (mg/kg of BW)	Alternate
Control	Water		Water
Morphine	Morphine	4.0	Saline
β-Casomorphin			
1	β-Casomorphin	1.25	Water
2	β-Casomorphin	2.5	Water
3	β-Casomorphin	5.0	Water
4	β-Casomorphin	10.0	Water

The group of rats conditioned with morphine, 4.0 mg/kg, which were assessed before, during, and after the rats conditioned with β-casomorphin were assessed, served as a standard to ensure that the procedures used to assess the effects of β-casomorphin would establish a CPP with an agent known to produce a CPP.

Measure, Data Reduction, and Statistics

The percentages of time spent on the putative side of conditioning on the baseline and test days were tabulated. Because the groups were constructed to have nearly equal baseline preferences, the baseline scores were not used in the formal data analyses.

Initial analyses revealed that rats' scores across tests did not vary significantly. Also, side of alley (grey vs. striped) was not a reliable source of variance and did not interact with other factors. Therefore, data were collapsed across tests by calculation of a mean test score for each rat. With these reductions of the data, the appropriate analysis was a one-way ANOVA of the groups' mean test scores. Mean scores of the control group were compared with the mean scores of each of the other groups using Student's t tests for independent measures.

RESULTS AND DISCUSSION

The mean baseline preference for the putative side of conditioning for all rats was 45.8 ± 1.75% ($\bar{X}$ ± SEM). The mean baseline preferences for the six groups ranged from 45.58 to 45.92%. Thus, our attempt to construct groups with roughly equal mean baseline preferences was successful.

The mean test scores are presented in Table 2. The ANOVA of that data yielded $F(5,78) = 5.06$, $P = .0005$. As expected, morphine produced a CPP, $t(34) = 2.31$, $P < .03$. None of the groups receiving a dose of β-casomorphin had mean preference scores that were reliably greater than those of controls. Thus, at least in the dose range assessed, β-casomorphin did not produce a reliable CPP, suggesting that these doses are not rewarding and thus do not have a high addiction liability.

TABLE 2. Proportion of time spent by rats on the putative side of conditioning across three tests of conditioned place preference.

Group	Dose	Time	SE
	(mg/kg of BW)	(%)	
Control		42.9[b]	4.41
Morphine	4.0	55.2[a]	2.92
β-Casomorphin			
1	1.25	42.3[b]	3.03
2	2.5	29.9[c]	4.21
3	5.0	41.3[b]	4.54
4	10.0	43.8[b]	5.12

[a,b,c]Means followed by the same superscripted letter do not differ ($P > .05$).

CONCLUSIONS

Negative results are always difficult to interpret. The data fail to indicate that β-casomorphin produced effects different than placebos. Small peptides such as β-casomorphin are probably digested in the gut, and the products are used as nutrients. Also, even though some small peptides may cross the blood brain barrier, they usually do not. Given these considerations and the results of this test, opioid peptides occurring in milk or milk products, when ingested, are not likely to have affective consequences similar to those of orally administered morphine. Consequently, the likely occurrence of β-casomorphin in milk products is not problematic. However, these results do not address the possibility that β-casomorphin might have effects in infants, for whom the blood brain barrier is not yet fully developed.

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