pose that the reward that supports lever pressing is mediated by a dopamine system while nose poking is supported by the activity of some other nondopaminergic substrate. This would account for the differential effects of α-flupenthixol on the two responses. No matter which hypothesis one adopts to explain these results, it remains the case that α-flupenthixol does not produce anhedonia.

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6. Each 5-μA current adjustment was maintained for 5 minutes, followed by a 15-second intertrial period during which no stimulation was available. A new trial was signaled by turning on a masking noise delivered through speakers inside each test chamber. For sessions involving nose poking, the positive hole was alternated after each 5-minute trial. This procedure was maintained throughout testing. Currents ranged in intensity from 0 to 40 μA.
7. α-Flupenthixol (1.0 ml per kilogram of body weight) was dissolved in 0.9 percent sodium chloride solution and injected intraperitoneally. The ability of α-flupenthixol to inhibit binding of [^{3}H]haloperiodol and [^{3}H]spiroperidol and its average clinical daily dose are virtually identical to that of pimozide, the neuroleptic used in most of the previous studies [I. Creese, D. R. Burt, S. H. Snyder, *Science* **192**, 481 (1976); J. E. Leysen, W. Gommeren, P. M. Laduran, *Biochem. Pharmacol.* **27**, 3077 (1978)].
8. To reduce the possibility that tolerance would develop after repeated injections of the drug, the test days were separated by 1 week. Self-stimulation rates were measured 1 and 2 days before each test day to ensure that performance had returned to normal levels. Self-stimulation thresholds on the day before testing remained stable over the entire experiment for nose poking and lever pressing. Differences observed on test days cannot, therefore, be attributed to shifts in baseline values over the course of the study.
9. A two-factor analysis of variance with repeated measures on both factors was computed for the arc sine–transformed data plotted in Fig. 1C. Response rates in both response conditions were reduced by the administration of α-flupenthixol [$F(2, 14) = 11.71$, $P < .005$)]. In addition, lever pressing was attenuated more than nose poking during the three drug trials [$F(1, 7) = 5.60$, $P < .05$)]. This difference in performance was consistent over all three doses of α-flupenthixol as revealed by the lack of a reliable dose × response interaction [$F(2, 14) = 2.12$, not significant].
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Bee Venom Enhances Guanylate Cyclase Activity

Abstract. *Bee venom and phospholipase A2 extracted from bee venom enhanced guanylate cyclase (E.C. 4.6.1.2) activity two- to threefold in rat liver, lung, heart, kidney, ileum, and cerebellum. Dose-response relationships revealed that bee venom at concentrations as low as 1 microgram per milliliter and phospholipase A2 at 1 microunit per milliliter caused a maximal enhancement of guanylate cyclase.*

Each year in the United States, nearly twice as many people die from hymenopterous insect bites (including bees, wasps, hornets, and yellow jackets) as from poisonous snake bites (*1*). The majority of these deaths appear to be due to severe systemic anaphylactic reactions, characterized by respiratory distress often followed by vascular collapse or shock. Bee venom is one of the best characterized of hymenopterous venoms and contains 0.1 to 1.5 percent histamine, two enzymes (phospholipase A_2 and hyaluronidase) and a series of toxic polypeptides (the hemolyzing mellitin, the neurotoxic apamin, and a mast cell degranulating peptide) (*2*). Mellitin, which makes up 50 percent of the dry weight of bee venom, is thought to be the main toxin of bee venom although practically all the effects of bee venom have been ascribed to its phospholipase activity (*2*). Mellitin, a known membrane-active peptide, has been shown to stimulate the activity of phospholipase A_2 (*3*) and heart microsomal guanylate cyclase (E.C. 4.6.1.2) activity (*4*). Since mellitin's effects may be mediated via activating phospholipase A_2, which in turn might activate guanylate cyclase activity, experiments were performed to examine whether phospholipase A_2, isolated from bee venom of *Apis mellifera* (honey bee), and the total honey bee venom itself have any influence on guanylate cyclase activity. With respect to possibility of bee venom affecting guanylate cyclase activity there is the recent demonstration (*5*) that α-toxins of the poisonous snakes krait (*Bungarus multicinctus*) and cobra (*Naja naja siamenis*) enhance the soluble form of guanylate cyclase in rat lung, spleen, and kidney. Both the total bee venom and the honey bee phospholipase A_2 enhanced guanylate cyclase activity in rat liver, lung, heart, kidney, ileum, and cerebellum suggesting that bee venom's mechanism of action is similar to that of the α-toxins of poisonous snakes.

Tissues used in these experiments were from Sprague-Dawley rats; they were homogenized and processed (*6*) to obtain the supernatant and particulate cell extracts after centrifugation at 37,000*g*. Guanylate cyclase was assayed (*6*) with the use of a reaction mixture consisting of 20 m*M* tris-HCl, *p*H 7.6; 4 m*M* $MnCl_2$; 2.67 m*M* cyclic guanosine monophosphate (GMP) (used to minimize destruction of ^{32}P-labeled cyclic GMP); a guanosine triphosphate (GTP) regenerating system (5 m*M* creatine phosphate and 11.25 units of creatine phosphokinase, E.C. 2.7.3.2); 100 μg of bovine serum albumin; 20 m*M* caffeine; and 1.2 m*M* (α-^{32}P)-labeled GTP, approximately 5×10^5 count/min. The enzyme preparations had 0.1 to 0.4 mg of protein. The cyclic [^{32}P]GMP formed was isolated by sequential chromatography on Dowex-50-H^+ and alumina (*6*). Reactions were conducted at 37°C. One unit of phospholipase A_2 is the amount that will hydrolyze 1.0 μmole of L-α-phosphatidylcholine to lysophosphatidyl choline and fatty acid per minute at *p*H 8.5 at 37°C. Sources of all other reagents have been reported (*6*). Each assay was conducted in triplicate, and each value in Table 1 and Fig. 1 was the mean ± the standard error of the mean in three separate experiments with three animals for each experiment each day ($N = 9$).

Honey bee venom and phospholipase A_2 extracted from honey bee venom enhanced soluble guanylate cyclase activity in various tissues (Table 1). Thus, phospholipase A_2 (1 μU/ml) and the bee venom (1 μg/ml) itself enhanced guanylate cyclase activity two- to threefold in rat liver, kidney, lung, heart, ileum, and cerebellum. Both bee venom and phospholipase A_2 increased guanylate cyclase activity more in the ileum and lung

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than the other tissues. Dose-response relationships on kidney cortex indicated that both bee venom and phospholipase A_2 increased guanylate cyclase activity to a similar extent, although the enhancement with phospholipase A_2 was always somewhat less than that seen with bee venom itself (Fig. 1). Half-maximal stimulation of guanylate cyclase activity was seen at 0.01 μg/ml with bee venom and at 0.1 μU/ml with phospholipase A_2. Increasing the concentration to milligrams per milliliter for bee venom and milliunits per milliliter for phospholipase A_2 caused no further enhancement of guanylate cyclase activity. Similar dose-response curves were seen with the other tissues utilized. At 37,000*g*, particulate guanylate cyclase activity makes up only 5 percent of the total guanylate cyclase activity. There was very little effect of bee venom or phospholipase A_2 on particulate guanylate cyclase activity (data not shown).

Bee venom in the present investigation was demonstrated to enhance guanylate cyclase activity in vitro. As was mentioned above, bee venom is composed of several polypeptides, two enzymes, and a small amount of histamine. The polypeptide mellitin is a membrane-active agent that has been reported to enhance particulate heart guanylate cyclase activity but did not have any effect on soluble guanylate cyclase activity (*5*) as was found with bee venom and phospholipase A_2 in the present investigation. Mellitin is known to activate endogenous phospholipase A_2 to produce lysolecithin and free fatty acids from *Escherichia coli* cytoplasmic membranes (*3*) and from cultured 3T3-4a mouse fibroblast cells (*7*). Since lysolecithin and free fatty acids have also been reported to enhance guanylate cyclase activity (*8*), one suggested mechanism for bee venom enhancement of guanylate cyclase in vitro would be that mellitin enhances phospholipase A_2 which, in turn, would increase fatty acids and lysolecithin to enhance guanylate cyclase activity. Whether this whole series of steps is necessary for bee venom enhancement of guanylate cyclase is unknown at present, but there is some evidence to suggest that the rate of hydrolysis of lecithin in the presence of mellitin is insufficient to produce enough lysolecithin to activate guanylate cyclase (*4*). Small amounts of phospholipase A_2 from bee venom, however, were capable of enhancing guanylate cyclase activity, suggesting that at least part of the guanylate cyclase activation observed secondary to bee venom may be due to a direct activation of guanylate cyclase by phospholipase A_2.

Phospholipase A_2 did not achieve the same maximal enhancement of guanylate cyclase activity in vitro as was seen with bee venom itself (Fig. 1), suggesting that something else in the bee venom was also activating guanylate cyclase activity. Histamine, which is only 0.1 to 1.5 percent of bee venom by weight (*2*) has been reported to increase cyclic GMP levels in various tissues (*9*). The higher maximal enhancement seen with bee venom itself as opposed to phospholipase A_2 enhancement of guanylate cyclase activity may possibly be due to the small amount of histamine in the total bee venom. Although dopamine and noradrenaline have been reported to be present in bee venom reservoirs in vivo, they are reportedly not present in the secreted dried venoms (*10*), and one would therefore not expect either of them to be the cause of the greater maximal enhancement of guanylate cyclase activity observed with bee venom in vitro.

The enhancement of guanylate cyclase activity by bee venom indicates that bee venom may have a mechanism of action similar to the α-toxins of the poisonous snakes krait and cobra that have recently been demonstrated (*5*) to enhance the soluble form of guanylate cyclase in rat lung, spleen, and kidney. Further investigation is necessary to determine whether the enhancement of guanylate cyclase activity is a general phenomenon of toxins produced by poisonous animals.

Table 1. The effect of honey bee venom and phospholipase A_2 extracted from honey bee venom on soluble guanylate cyclase activity of various tissues. The supernatant of each of the respective tissues was assayed as described in the text. Each value represents the mean ± S.E.M. of triplicate samples determined in three separate experiments with three animals for each experiment ($N = 9$). Bee venom was tested at 1 μg/ml; phospholipase A_2 was tested at 1 mU/ml.

Tissue	Cyclic GMP (pmole per milligram of protein per 10 minutes)		
	No addition	Bee venom*	Phospholipase A_2*
Heart	156 ± 9	349 ± 16	322 ± 13
Liver	279 ± 12	642 ± 20	598 ± 19
Kidney	288 ± 11	651 ± 22	603 ± 21
Lung	1620 ± 21	4833 ± 27	4699 ± 32
Cerebellum	489 ± 13	1009 ± 19	988 ± 18
Ileum	643 ± 17	2003 ± 31	1833 ± 23

*Significant at $P < .001$ compared to controls by Student's *t*-test for unpaired values.

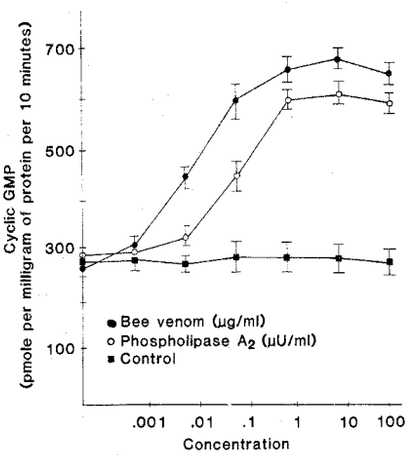

Fig. 1. Dose-response relationships of bee venom and phospholipase A_2 on kidney cortex guanylate cyclase activity in vitro. One unit of phospholipase A_2 is the amount that will hydrolyze 1.0 μmole of L-α-phosphatidylcholine to lysophosphatidyl choline and fatty acid per minute at *p*H 8.5 at 37°C. Each value (point) is the mean ± S.E.M. of triplicate samples determined in three separate experiments with three animals for each experiment ($N = 9$). The values of 0.01 μU/ml for bee venom and at 0.1 μU/ml (and higher concentrations) for phospholipase A_2 were significant at $P < .001$, compared to the controls by the Student's *t*-test for unpaired values.

The enhancement of guanylate cyclase activity by bee venom indicates that bee venom may have a mechanism of action similar to the α-toxins of the poisonous snakes krait and cobra that have recently been demonstrated (*5*) to enhance the soluble form of guanylate cyclase in rat lung, spleen, and kidney. Further investigation is necessary to determine whether the enhancement of guanylate cyclase activity is a general phenomenon of toxins produced by poisonous animals.

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