Electrophysiological Interactions of Isomers of Cyclazocine with the Phencyclidine Antagonist Metaphit in Rat Cerebellar Purkinje Neurons

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Metaphit, 1-(1-(3-isothiocyantophenyl) cyclohexyl) piperidine, an analog of phencyclidine (PCP) has been shown previously to selectively block PCP receptors and to irreversibly antagonize the depressant effect of PCP in cerebellum. In this study, we examined the electrophysiological interactions of metaphit and naloxone with stereoisomers of cyclazocine, an agent known to have analgesic and psychotomimetic activity in behavioral studies, effects that have been ascribed to opiate and PCP receptor activity. A dose-dependent and reversible slowing of Purkinje neuron discharge was seen with local application of (+)- or (-)-cyclazocine. We found that the blockade of (-)-cyclazocine effects required both high doses of naloxone and the presence of metaphit, whereas the responses to (+)-cyclazocine were blocked by metaphit alone on most cerebellar Purkinje neurons. These findings suggest that the depressant reaction of (+)-cyclazocine in cerebellar Purkinje neurons is primarily mediated through PCP receptors. (-)-Cyclazocine responses, on the other hand, appear to be due to activity at both PCP and κ opioid receptors.

On the basis of physiological and behavioral studies, the existence of multiple opioid receptors was first proposed by Martin and his associates (Gilbert and Martin, 1976; Martin, 1981; Martin et al., 1976). The prototypic opiates, such as morphine, elicit analgesia and sedation via a μ receptor (Pasternak et al., 1980). While benzomorphans such as cyclazocine and ketocyclazocine also elicit such effects, they show little cross tolerance with morphine and are antagonized by much higher doses of naloxone than are needed to block the effects of morphine (Hutchison et al., 1975; Kosterlitz et al., 1981). On the basis of these and other findings, it was postulated that cyclazocine and ketocyclazocine are antagonists at the μ receptor and full agonists at a second, κ , opioid receptor (Martin, 1981).

In addition to these effects, cyclazocine and *N*-allylnormetazocin (SKF-10047) have been shown to elicit dysphoric and psychotomimetic effects in man and delirium in dogs (Jansinski et al., 1968; Keats and Telford, 1964; Martin et al., 1965, 1976; Vaupel, 1983). Since these effects are not mimicked by either morphine or ketocyclazocine, and since they are not antagonized

even by very high doses of naloxone (Vaupel, 1983; Zukin and Zukin, 1983), a third, σ , receptor has also been postulated. The σ -mediated behavioral effects of cyclazocine (Brady et al., 1982; Cowan, 1981; Gilbert and Martin, 1976; Martin et al., 1965; Shannon, 1983; Slifer and Balster, 1983) are of particular interest since these are similar to and generalize with the non-soporific response to the dissociative anesthetic, phencyclidine (PCP) (Herling and Woods, 1981; Teal and Holtzman, 1980; Vaupel, 1983; Vaupel and Jansinski, 1979). Since PCP, like cyclazocine, is a potent psychotomimetic in man (Bakker and Amini, 1961), further exploration of the similarities between these 2 substances was undertaken. Several electrophysiological studies from our laboratory have provided additional data on the relationship of PCP and cyclazocine. These studies were carried out in rat cerebellum since significant populations of κ and PCP receptors are present in this region (Itzhak et al., 1985; Largent et al., 1984; Zukin and Zukin, 1981, 1983; Zukin et al., 1983) and since cerebellar Purkinje neurons can be readily identified during acute recording (Eccles et al., 1967). The local application of cyclazocine or PCP by pressure ejection slowed the discharge of cerebellar Purkinje neurons. While the effects of PCP application were insensitive to naloxone, the cyclazocine responses comprised both a naloxone-sensitive and a naloxone-insensitive component (Kim et al., 1985). However, the observed partial antagonisms of cyclazocine effects were transient and required high doses of naloxone (>1.0 mg/kg). These data indicated that at least 2 mechanisms may mediate the electrophysiological effects of (-)-cyclazocine in cerebellum. It was suggested that the naloxone-sensitive cyclazocine effects may be mediated via κ /opiate receptors since the density of μ receptors in albino rat cerebellum is extremely low (Antweh and Kuhar, 1977; Hiller et al., 1973; Pert et al., 1976; Snyder et al., 1974), and since the high doses of naloxone used in that study were in the range previously reported to block κ mechanisms (Harris, 1980; Iwamoto, 1981; Martin et al., 1976; Romer et al., 1980; Teal and Holtzman, 1980; Zukin and Zukin, 1981). On the other hand, the naloxone-insensitive effects of cyclazocine were phenomenologically similar to those caused by PCP. Indeed, our electrophysiological studies, which suggest that (-)-cyclazocine is the more potent stereoisomer in cerebellum (Kim et al., 1985), are consistent with previous reports that (-)-cyclazocine is more potent than the (+) isomer both in discriminative stimulus studies (Shannon, 1982) and in competing for PCP or dextroamphetamine binding sites (Mendelsohn et al., 1984; Sethy and McCall, 1984; Zukin, 1982). Even so, it cannot be concluded that the naloxone-insensitive effect of cyclazocine is mediated by a σ /PCP receptor mechanism without evidence that PCP receptor blockers also antagonize this response.

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Figure 1. Lack of antagonism of (–)-cyclazocine by locally applied metaphit. Here and in Figures 2–6, (–)-cyclazocine is indicated as CYC. A–C, Ratemeter records from 3 different Purkinje cells. In each case, the control responses of (–)-cyclazocine (solid line) or PCP (dotted line, cell C) are shown on the left side of the arrow, and the responses after metaphit are shown on the right. Arrows represent local applications of 0.2% metaphit at 5–10 psi for 30–90 sec. Such applications abolish spontaneous discharge. Recovery of firing usually takes 5–20 min, after which (–)-cyclazocine-induced responses are unaffected (A–C), whereas PCP responses (C) are blocked. In this and all succeeding ratemeter records, the duration of drug ejection is indicated by the bar overlying the trace, and the ejection pressure (in psi) is given by the number after the drug.

We have recently reported that metaphit [1-(1-(3-isothiocyanatophenyl) cyclohexyl) piperidine], which has been found to acylate PCP receptors irreversibly (Rafferty et al., 1985), completely antagonizes the electrophysiological action of PCP in the cerebellum (Wang et al., 1985, 1986a). In the study reported here, we investigated the relative roles of PCP and κ receptor mechanisms in the mediation of the electrophysiological actions of cyclazocine in cerebellum. The interactions of metaphit with (+)-cyclazocine and (–)-cyclazocine on cerebellar Purkinje neurons were investigated both before and after the administration of high doses of naloxone. The data reported here suggest that the electrophysiological effects of cyclazocine on cerebellar Purkinje neurons have at least 2 components, one of which is κ /opiate-like in that it is sensitive to high doses of naloxone and one of which is PCP-like in that it is sensitive to metaphit.

Materials and Methods

Male Sprague-Dawley rats (200–350 g) were anesthetized with urethane (1.25 g/kg, i.p.), intubated, and placed in a stereotaxic frame. The skull and dura overlying the cerebellum were removed, and the cerebellar surface was covered with 2.0% agar. The cisterna was opened at the foramen magnum to reduce brain pulsations. Body temperature was monitored with a thermistor probe and maintained at 37°C with a heating pad.

Extracellular action potentials were recorded from single cerebellar Purkinje neurons in lobules VI and VII of the cerebellar vermis through the 5 M NaCl-filled barrel of 3 or 4 barrel micropipettes that were constructed as previously described (Palmer et al., 1980). Neuronal activity was filtered, monitored on an oscilloscope, and then converted to constant voltage pulses using a window discriminator. The pulses were integrated over 1 sec epochs by a ratemeter and were displayed on a strip-chart recorder. Cerebellar Purkinje neurons were identified by their characteristic discharge pattern of simple and complex spikes (Eccles et al., 1967).

Drugs were applied from 2 or 3 barrels of the micropipette by pressure ejection as previously described (Palmer et al., 1980). Pressure applications were regulated by a pneumatic valve (1–35 psi), and the timing of drug pulses was controlled by crystal clock circuit. Previous studies have shown that drug administration by this technique is reproducible

and is linearly related to pressure and time of ejection (McCaman et al., 1977). To be considered valid, all responses to pressure-ejected agonists were required to show dose dependency, reversibility and reproducibility. Previously described controls were used to test for local anesthesia, pH effects, drug specificity, and pressure artifacts (Palmer, 1982). The drug solutions that were pressure ejected were 1 mM (–)-cyclazocine, 1 mM (+)-cyclazocine, 1 mM phencyclidine [1-(1-phenylcyclohexyl) piperidine HCl; PCP]; 0.5 mM naloxone HCl, and 5.5 mM metaphit. All drugs used in local applications were made up in 165 mM NaCl, pH 7.0, except as noted. Naloxone HCl (Narcan; Dupont Pharmaceuticals) was also administered subcutaneously (s.c.) or intraperitoneally (i.p.) at 1, 2, 5, or 10 mg/kg.

Ratemeter records were analyzed for changes in neuronal discharge caused by local drug application as previously described (Palmer and Hoffer, 1980). The data were digitized and quantified by computer analysis to indicate the percentage of depression or excitation of neuronal activity caused by drug application. Previous reports from our laboratory have evaluated the validity of this analytic approach to quantitate responses of neurons to microadministration of drugs (Freedman et al., 1975; Palmer et al., 1980). Using these techniques, drug responses can be evaluated independently of changes in background discharge, and apparent antagonism due to changes in release of drug from the pipette can be minimized (Freedman et al., 1975).

Results

Local application of (–)-cyclazocine, administered by pressure ejection, reversibly slowed Purkinje neuron discharge (Fig. 1, A–C, left-hand side of arrows). Metaphit, when applied locally (vertical arrows) with ejection parameters that readily block PCP-induced responses (Wang et al., 1985, 1986a) did not antagonize (–)-cyclazocine-induced inhibitions of most Purkinje neurons. Thus, the (–)-cyclazocine-elicited depression from 12 of 14 neurons was unaltered by 30–90 sec applications of metaphit (Table 1). There was less than a 50% antagonism of (–)-cyclazocine effects on the remaining 2 cells. PCP applied alternately with CYC to 3 neurons produced effects that were completely blocked by doses of metaphit that left (–)-cyclazocine responses unchanged (Fig. 1C).

In order to further differentiate between PCP and κ receptor-

Figure 2. Weak and transient blockade of (–)-cyclazocine by i.p. naloxone. The control responses (*A*) show (–)-cyclazocine-induced depressions of one P neuron. After parenteral naloxone application (*B* and *C*, 6 mg/kg, i.p.), (–)-cyclazocine responses are only partially and transiently antagonized.

mediated effects, the interaction of (–)-cyclazocine with high doses of naloxone was studied. Naloxone, when administered at doses of 6–10 mg/kg, i.p. (Fig. 2, *B*, *C*) or subcutaneously (Fig. 3, *B*, *C*), or when applied locally for several minutes by pressure ejection, caused weak and transient antagonisms of (–)-cyclazocine-induced inhibitions in 4 of 9 neurons. The cyclazocine responses were reduced by less than 50% and the antagonisms to systemic naloxone lasted less than 5 min (Figs. 2, 3). The effects of (–)-cyclazocine on the additional 5 neurons were not antagonized by naloxone (Table 1).

Although the electrophysiological effects of (–)-cyclazocine are not completely blocked by treatment with either metaphit or naloxone alone, the observed (–)-cyclazocine-induced depressions of neuronal activity may be mediated partially by a naloxone-sensitive mechanism and partially by activation of a metaphit-sensitive site. If this is true, then combinations of these 2 receptor antagonists might be expected to block the effects of locally applied (–)-cyclazocine. Two different proto-

cols were used to test this conjecture: (1) locally applied metaphit after parenteral naloxone and (2) locally applied naloxone after local metaphit. The combined administration of naloxone and metaphit completely antagonized the effects of locally applied (–)-cyclazocine on 10 of 12 Purkinje neurons tested (Table 1). Two examples of the blockade using the first protocol are shown in Figure 4. Parenteral naloxone, 8 mg/kg, was given prior to the leftmost ratemeter trace in each case. Although (–)-cyclazocine still produced a potent effect after the naloxone treatment, this response was blocked after subsequent treatment with metaphit (Fig. 4, right-hand side of arrows).

One problem with first protocol is that both the high-dose parenteral naloxone and metaphit applications are quite long lasting. Thus, recovery of agonist effect cannot be easily demonstrated. Hence, the second protocol, using locally applied naloxone, was also used (Figs. 5 and 6). Following local applications of metaphit (vertical arrows), pressure-ejected naloxone completely and reversibly blocked the (–)-cyclazocine-induced depressions of Purkinje neurons. These drug interactions were reproducible on each neuron studied (Figs. 5, *C*, *D*, and 6, *D*, *E*).

A markedly different antagonist sensitivity was observed for the (+)-isomer of cyclazocine. Although totally insensitive to naloxone, the depressant actions of (+)-cyclazocine were completely antagonized by metaphit alone in 11 of 14 neurons tested (Fig. 7, *A–D*; Table 2). The blockade of the depressant effects of (+)-cyclazocine on cerebellar Purkinje neurons began after the application of metaphit and did not recover during the 1 hr testing period used for each cell. The (+)-cyclazocine induced depressions from 3 of 14 Purkinje neurons were insensitive to the metaphit treatment (Fig. 7*E*).

Table 1. Blockade of (–)-cyclazocine by metaphit and/or naloxone in cerebellar Purkinje neurons

Antagonist	Blockade		
	None	Weak (<50%)	Strong (>90%)
Metaphit	12 ^a	2	0
Naloxone	5	4	0
Metaphit + naloxone	2	0	10

^a Number of neurons.

Figure 3. Weak and transient blockade of (-)-cyclazocine by s.c. naloxone. *A*, Control (-)-cyclazocine responses. *B* and *C*, After s.c. injection of naloxone, 10 mg/kg, (-)-cyclazocine responses were partially and transiently antagonized.

Discussion

Similar to a previous study from this laboratory (Kim et al., 1985), we found that responses to (-)-cyclazocine were partially, although transiently, antagonized by high doses of the opiate antagonist, naloxone. We also previously reported that the naloxone-insensitive effects of (-)-cyclazocine in cerebellum were similar to those caused by PCP in that brain area. In the present

investigation, we also studied the sensitivity of cyclazocine-induced responses to metaphit, an isothiocyanate derivative of PCP that has been reported to form covalent bonds with PCP receptors selectively (Rafferty et al., 1985). While the electrophysiological effects of the (+) isomer of cyclazocine were blocked by local metaphit application, responses to (-)-cyclazocine were either unaltered or were only partially antagonized by this putative irreversible PCP receptor antagonist. These data could

Figure 4. Blockade of cyclazocine-induced depression by local metaphit after parenteral naloxone. P cells (*A* and *B*) from 2 different animals are illustrated. The control records (*left of arrow*) show responses to (-)-cyclazocine after parenteral naloxone. Following local metaphit application (*vertical arrow*, 5-10 psi, 45 sec) and recovery of discharge, responses to (-)-cyclazocine (*right of arrow*) were completely antagonized.

Figure 5. After, but not before, local metaphit application, (-)-cyclazocine was reversibly blocked by local naloxone. Control responses (*A*) represent (-)-cyclazocine effects during local naloxone application. After applying metaphit (vertical arrow at end of *A*, 18 psi, 180 sec), (-)-cyclazocine effects were gradually antagonized by a second application of naloxone (*B*). Discontinuing naloxone (*C*) permits recovery of (-)-cyclazocine effects. Application of naloxone for a third time again blocks (-)-cyclazocine-induced slowing (*D*).

Figure 6. Reversible blockade of (-)-cyclazocine by local naloxone in the presence of metaphit. *A*, Control (-)-cyclazocine responses in the presence of naloxone. *B*, and *C*, After local metaphit application (vertical arrow at end of *A*, 15 psi, 260 sec), (-)-cyclazocine's depressant effects gradually disappeared. *D*, Discontinuing naloxone led to the recovery of (-)-cyclazocine responses. *D* and *E*, Second application of naloxone locally again causes blockade of (-)-cyclazocine-induced depressions.

Figure 7. Interactions of (+)-cyclazocine (+cyc), and metaphit on cerebellar P cells. A–E, Ratemeter records from 5 different cells. Left-most traces illustrate control (+)-cyclazocine-induced depressions. After application of metaphit (vertical arrows), (+)-cyclazocine was again tested in the right-most traces. Note complete antagonism in cells A–D and no blockade in cell E.

suggest that (+)- but not (–)-cyclazocine had a PCP-like mechanism of action. This is consistent with the behavioral observations (Solomon et al., 1981) that (+)- but not (–)-cyclazocine has discriminative properties in rhesus monkeys that are similar to ketamine, a PCP analog. However, (–)-cyclazocine does cause PCP-like stimulus effects in other animal species, such as rats (Shannon, 1982), pigeons (McMillan, 1982), and squirrel monkeys (Holtzman, 1982). Indeed, the effects of (–)-cyclazocine could be completely antagonized in this study by the concomitant administration of both metaphit and a high dose of naloxone.

Even though (–)-cyclazocine-induced depressions of Purkinje neurons were not blocked by either naloxone or metaphit alone, the observation that these responses were blocked by high doses of naloxone in the presence of metaphit suggests that (–)-cyclazocine interacts with at least two classes of receptors in cerebellum at the doses studied. The metaphit-sensitive component of the response may represent actions of cyclazocine at the PCP/ σ site. Not only has metaphit been reported to bind to PCP receptors in the brain irreversibly (Rafferty et al., 1985), but we have also previously shown that metaphit specifically and irreversibly blocks the electrophysiological effects of PCP in the cerebellum (Wang et al., 1986a) and hippocampus (Wang et al., 1986b). Furthermore, in contrast to findings on the hippocampus, where only 60% of the PCP receptors are metaphit sensitive, our preliminary neurochemical evidence indicates that nearly 100% of the PCP binding sites in cerebellum are blocked after treatment with 10 μ M metaphit (Wang et al., 1985). Thus, the actions of both isomers of cyclazocine appear to involve this metaphit-sensitive PCP mechanism in the cerebellum. The interactions of cyclazocine with PCP mechanisms in other brain

regions, such as hippocampus, may prove more complex.

Our observation that the electrophysiological effects of (–)-cyclazocine in the cerebellum are partially mediated by a naloxone-sensitive mechanism is consistent with the behavioral findings of Teal and Holtzman (1980). They reported that the effects of cyclazocine in the rat discriminative stimulus assay are only partially reversed by naltrexone, another opiate antagonist. Furthermore, (–)-cyclazocine is over 15 times more potent than morphine as an analgesic in the phenylquinone (PPQ) antinociceptive assay in mice. Similar to the data reported here, the analgesic action of (–)-cyclazocine can be blocked only by high doses of naloxone in the PPQ assay. In contrast, cyclazocine has no analgesic effect in the PPQ assay at doses up to 1.0 mg/kg, which is more than 70 times greater than that of (–)-cyclazocine in this assay. It is known from binding studies that (–)-cyclazocine has high affinity for both μ and κ opioid receptors as well as for PCP receptors (Chang et al., 1980; Zukin and Zukin, 1981). However, there are few μ receptors in cerebellum (Kuhar et al., 1973), and the doses of naloxone required to block κ receptors are much higher than those needed for μ antagonism.

Table 2. Blockade of (+)-cyclazocine by metaphit in cerebellar Purkinje neurons

Antagonist	Blockade		
	None	Weak (<50%)	Strong (>90%)
Metaphit	3 ^a	0	11

^a Number of neurons.

(Kosterlitz et al., 1981). Thus, the component of the (–)-cyclazocine response in cerebellum that is sensitive to high doses of naloxone might represent a κ opiate receptor interaction.

Our observation that both opiate and PCP receptors must be inactivated in order to antagonize the effects of (–)-cyclazocine in the cerebellum suggests that both κ and PCP mechanisms are involved in the Purkinje neuron responses to (–)-cyclazocine. Thus, the blockade of either receptor type alone is insufficient for antagonizing these responses. Indeed, Slifer and Balster (1983) have argued that the apparent stereoselectivity for (–)-cyclazocine in many studies has been due to contamination of the results with the activation of κ receptors or with other naloxone-sensitive opiate mechanisms. Further studies will be required to determine if there are complex interactions between κ and PCP/ σ mechanisms in addition, perhaps, to influences of other neurotransmitters in this brain area that acutely compensate for the pharmacological antagonism of either receptor site.

In conclusion, our data suggest that while (–)-cyclazocine has activity at both PCP and κ mechanisms in cerebellum, (+)-cyclazocine's depressant effects are probably mediated primarily through activation of the metaphit-sensitive PCP receptor. Determination of the extent, however, to which such interactions in cerebellum or elsewhere underlie the behavioral actions of (+)- and (–)-cyclazocine awaits further pharmacological experiments involving recordings in freely moving, unanesthetized animals.

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