Pharmacology Biochemistry and Behavior, Vol. 63, No. 1, pp. 185–192, 1999
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0091-3057/99/$–see front matter

PII S0091-3057(98)00267-6

Behavioral Effects of Family-Selective Inhibitors of Cyclic Nucleotide Phosphodiesterases

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Received 24 April 1998; Revised 15 October 1998; Accepted 27 October 1998

O'DONNELL, J. M. AND S. FRITH. *Behavioral effects of family-selective inhibitors of cyclic nucleotide phosphodiesterases.* PHARMACOL BIOCHEM BEHAV **63**(1) 185–192, 1999.—The effects of family selective inhibitors of phosphodiesterase (PDEl, PDE2, PDE3, PDE4, and PDE5) on the behavior of rats under either a differential-reinforcement-of-low-rate (DRL) 72-s schedule or a variable-interval (VI) 30-s schedule were determined; previous work has shown that antidepressant drugs increase reinforcement rate under long DRL schedules. The PDE4-selective inhibitor rolipram (0.03–0.1 mg/kg) reduced response rate and increased reinforcement rate under the DRL schedule in a dose-dependent manner; similar effects were observed with the tricyclic antidepressant drug desipramine (3–10 mg/kg). Both of these drugs produced biphasic effects on behavior maintained under the VI schedule, increasing response rate at the lower doses tested (rolipram: 0.003 mg/kg; desipramine: 0.03 mg/kg) and decreasing response rate at higher doses (rolipram: 0.1 mg/kg; desipramine: 0.3–18 mg/kg). Of the other PDE inhibitors tested, only the PDE5-selective inhibitor zaprinast (10 mg/kg) produced an antidepressant-like effect on DRL behavior. However, in contrast to the biphasic effects of rolipram and desipramine on VI behavior, zaprinast produced monotonic decreases in response rate (10–30 mg/kg). The PDE2-selective inhibitor trequinsin produced biphasic effects on response rate under the VI schedule, increasing rates at low doses (3–5.6 mg/kg) and decreasing rates at higher doses (18–30 mg/kg). Trequinsin also reduced response rate under the DRL schedule (30 mg/kg); however, the reduction in response rate was not accompanied by increased reinforcement rate. The PDE3-selective inhibitor milrinone (1–10 mg/kg) tended to increase response rates under both schedules while the PDE1-selective inhibitor vinpocetine did not affect behavior at the dose range tested (1–30 mg/kg). These findings suggest that inhibition of PDE4 results in a rather unique pattern of behavioral effects, most notably an antidepressant-like effect on DRL behavior. It remains to be determined if a similar effect produced by zaprinast also implicates PDE5 in the mediation of antidepressant activity or represents an effect of this drug on PDE4 activity at high doses. © 1999 Elsevier Science, Inc.

Adenylyl cyclase Antidepressant drugs Behavior Desipramine Milrinone Phosphodiesterase Rolipram Trequinsin Vinpocetine Zaprinast

CYCLIC nucleotide phosphodiesterases (PDEs) comprise at least ten families of enzymes that hydrolyze the cyclic nucleotide second messengers cyclic AMP and cyclic GMP. To date, more than 30 variants belonging to these enzyme families have been identified (1,3,18,46). This great heterogeneity results from distinct gene products, different initiation sites, and splice variants. These diverse PDE enzymes provide the only known pathway for the enzymatic inactivation of the cyclic nucleotide second messengers, making them important components of signal transduction mechanisms. The classification of the PDEs into distinct families is based on substrate preference, sensitivity to modulators of activity (e.g., Ca^{++}/calmodulin, cyclic GMP), and structural homology (18,46). In several instances, PDEs have been shown to be regulated, both at the expression level as well as by posttranslational modification (41,47,49). Further, it appears that particular PDE variants may exhibit a unique subcellular localization or association with a particular receptor-coupled cyclase (4,42,56). This has led to the suggestion that inhibition of a particular family of PDE may result in a unique pattern of physiological and behavioral effects (14).

Of the seven PDE families, PDE4 has proven to be of particular importance in neuropsychopharmacology. Initial work by Wachtel and co-workers showed that rolipram, a selective

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inhibitor of PDE4, produced a characteristic pattern of effects including hypothermia, hypoactivity, and increased grooming and head twitching in mice (11,35,51,52). Similar effects were reported for a number of other inhibitors of PDE4; the potency with which the inhibitors produce these behavioral and physiological effects is correlated with the potency with which they inhibit the binding of ^{3}H-rolipram in the brain, in vivo (35). Further study of rolipram and other PDE4-selective inhibitors showed them to increase the acoustic startle response in rats, enhance morphine-induced antinociception, enhance retention in a passive avoidance task, and produce discriminative stimulus effects (16,22,30,33,36,50). In addition, rolipram and related drugs have been shown to produce antidepressant-like effects in a number of preclinical tests. These drugs suppress muricide in olfactory-bulbectomized rats, reduce the time of immobility of rats in the forced-swimming test, and reduce response rate and increase reinforcement rate of rats under a differential-reinforcement-of-low-rate (DRL) schedule (19,24,31). The antidepressant effects of selective inhibitors of PDE4 are supported by the results of a number of clinical studies (7,9,13,38,58). Overall, these findings have led to the speculation that inhibitors of PDE4 might represent a new class of antidepressant drugs, and that dysfunction in the regulation of PDE4 might contribute to the pathophysiology of depression (53).

In an earlier study, it was found that rolipram, Ro 20-1724, ICI 63,197, and CP 76,593, all selective inhibitors of PDE4, reduce response rates and increase reinforcement rates of rats under a DRL 72-s schedule (24). The results of lesion and antagonism experiments suggest that effects of rolipram are not due to actions on noradrenergic terminals or beta-adrenergic receptors, but most likely depend on postreceptor mechanisms. The results of other studies support such an interpretation (16,19,54). However, it has not been determined that the antidepressant-like behavioral effects of PDE4 inhibitors are unique among the family-selective inhibitors of PDE. Inhibitors of each of the PDE families are capable of increasing cyclic nucleotide concentrations. It is not clear whether antidepressant-like effects can be produced by inhibitors of other PDE families as a consequence of their effects on cyclic AMP and GMP concentrations, or whether the unique neuroanatomical distribution and subcellular localization of PDE4 makes the effects of its inhibitors unique.

It has been shown that PDE4 is the enzyme that hydrolyzes cyclic AMP formed by stimulation of beta-adrenergic receptors in the rat cerebral cortex (4,56); these receptors have been shown to be involved in the neurochemical and behavioral effects of proven antidepressant drugs (10,23,25,26,29). However, because other PDE families, most notably PDE1 and PDE2, have been shown to be present in rat brain (21), it remains to be determined whether family-selective inhibitors of the other PDEs share behavioral effects with PDE4 inhibitors. To address this question, the effects of family selective inhibitors (37) of PDE1 (vinpocetine), PDE2 (trequinsin), PDE3 (milrinone), PDE4 (rolipram), and PDE5 (zaprinast), as well as the proven antidepressant desipramine, on the behavior of rats under a DRL 72-s schedule were assessed. In addition, the effects of these drugs on behavior under a variable interval (VI) 30-s schedule were examined. Behavior maintained under this schedule has been shown to be sensitive to both rate-increasing and rate-decreasing effects of drugs; this permitted determination of whether stimulant actions or behavioral depressant effects of the PDE inhibitors contributed to their effects on DRL behavior.

METHOD

Subjects

Male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 350–450 g, were provided free access to laboratory chow and housed individually in polycarbonate cages in a room maintained at a constant temperature and humidity and illuminated 12 h per day. Access to water was restricted to a 1-h period following behavioral testing and was available as reinforcers during test sessions.

Behavioral Apparatus

Coulbourn Model E10-10 operant chambers were enclosed in sound-attenuating chambers equipped with fans that provided ventilation and masking noise. Each chamber contained two levers—a water access port, and a houselight. A downward force equivalent to 15 g (0.15 N) operated the right lever, constituting a response; responses on the left lever had no programmed consequence. When the schedule contingencies were met, a dipper was raised to the water access port, providing 0.02 ml of water, constituting a reinforcer. Behavioral responses were recorded and schedule events controlled by a PDP 11/73 computer using a LABLINC interface (State Systems, Kalamazoo, MI) and the SKED-11 operating system (44).

Behavioral Testing

Rats were initially trained under an alternative fixed-ratio 1-min, fixed-time 1-min schedule. Following acquisition of lever-pressing behavior, rats were trained to respond under either a DRL 18-s or VI 15-s schedule. As training progressed, the schedule contingencies were increased so that rats were trained under a terminal schedule of either DRL 72-s or VI 30-s. After 6–8 weeks, when performance under the schedules stabilized, groups of 6–12 rats were used to determine the effects of the PDE inhibitors and desipramine on behavior under the DRL 72-s and VI 30-s schedules. During the determination of the dose–response functions, rats were tested Monday–Friday. Drugs were administered on Tuesdays and Fridays; Thursdays served as noninjected control days. Each dose of each drug, as well as its vehicle, was administered at least twice in a mixed order.

Drugs

The PDE1-selective inhibitor vinpocetine, the PDE3-selective inhibitor milrinone, the PDE4-selective inhibitor rolipram, the PDE5-selective inhibitor zaprinast, and the tricyclic antidepressant desipramine were obtained from commercial sources (Research Biochemicals, Natick, MA; Sigma Chemicals, St. Louis, MO). The PDE2-selective inhibitor trequinsin was provided as a gift by Hoechst Celanese (Somerville, NJ). The PDE inhibitors were suspended in a 1% Tween 80/0.9% saline solution. Desipramine was dissolved in 0.9% saline. The drugs were administered IP in a volume of 1 ml per kg body weight 30 min prior to behavioral testing. Doses are expressed in terms of the free bases of the compounds.

Data Analysis

Data from dose–response functions were converted to the percentage of each rat's individual control response or reinforcement rates. Control values for a dose–response determination were the median of response or reinforcement rates for

Thursdays (i.e., the noninjected control day) during the period of time the data were collected. Data were analyzed by analysis of variance; individual comparisons were made using Newman–Keuls tests (55). Effects were considered statistically significant at $p < 0.05$.

RESULTS

Effects of the PDE1-Selective Inhibitor Vinpocetine

At the dose range tested, 1–30 mg/kg, vinpocetine did not affect the behavior of rats maintained under either the VI 30-s schedule, $F(4, 32) = 0.22$, or the DRL 72-s schedule, $F(4, 20) = 0.88$ and 1.67, for response and reinforcement rate, respectively (Fig. 1).

Effects of the PDE2-Selective Inhibitor Trequinsin

At doses of 1–10 mg/kg, trequinsin did not affect response or reinforcement rate under the DRL 72-s schedule (Fig. 2). At a dose of 18 mg/kg, trequinsin reduced response rate under the DRL 72-s schedule, $F(4, 36) = 26.32$, $p < 0.01$; this reduction in response rate was not accompanied by a change in average reinforcement rate, $F(4, 36) = 0.89$. In the initial animals to which it was administered, 30 mg/kg trequinsin completely inhibited responding under the DRL schedule; for this reason, the effect of this dose was not tested in the group as a whole.

Trequinsin produced biphasic effects on the behavior of rats under the VI 30-s schedule, $F(6, 66) = 20.49$; $p < 0.01$ (Fig. 2). Administration of doses of 3 or 5.6 mg/kg increased response rate, while administration of 18 or 30 mg/kg reduced response rate.

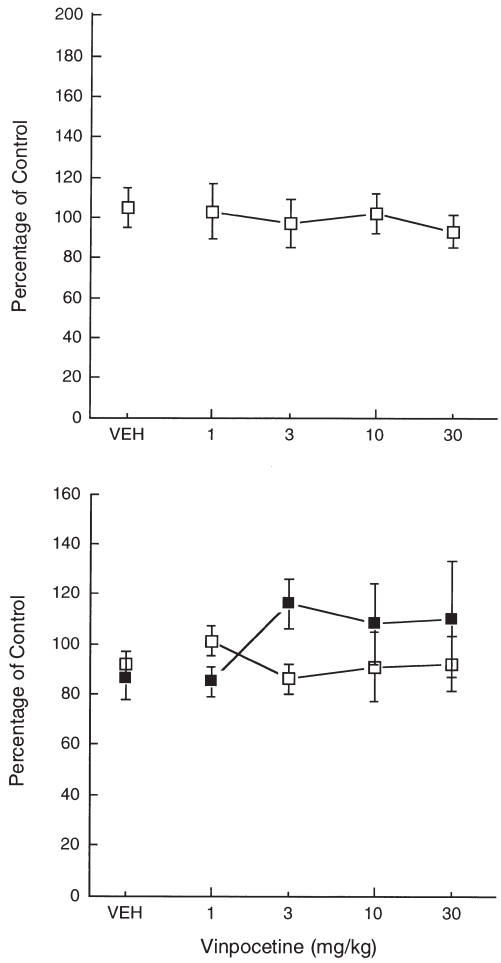

FIG. 1. The effects of the PDE1-selective inhibitor vinpocetine on response rate of rats under a VI 30-s schedule (upper panel) or on response rate (open squares) and reinforcement rate (filled squares) of rats under a DRL 72-s schedule (lower panel). Points represent the means ± SE for nine (VI) or six (DRL) rats expressed as a percentage of control performance. Control responses per minute (mean ± SD) under the VI schedule = 17.6 ± 7.9. Control responses and reinforcers per hour under the DRL schedule = 82 ± 14 and 11 ± 5, respectively.

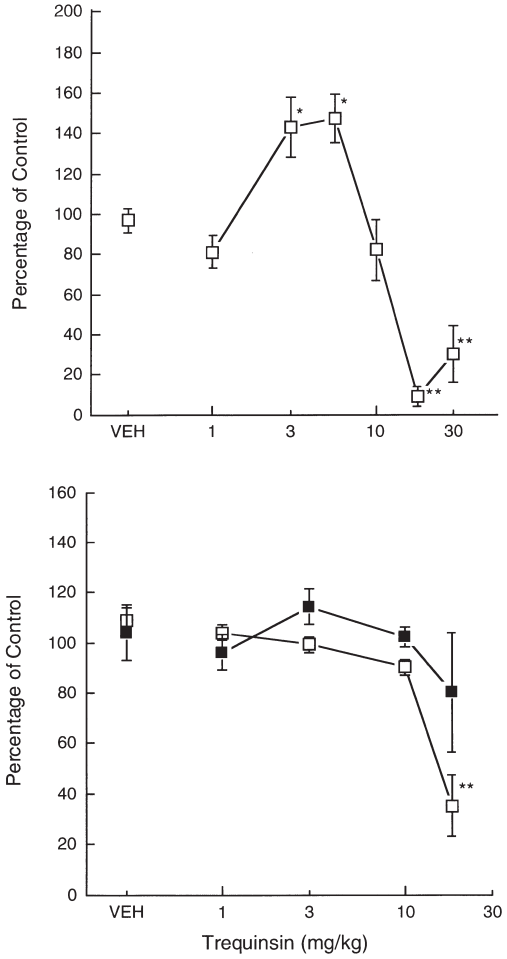

FIG. 2. The effects of the PDE2-selective inhibitor trequinsin on response rate of rats under a VI 30-s schedule (upper panel) or on response rate (open squares) and reinforcement rate (filled squares) of rats under a DRL-72-s schedule (lower panel). Points represent the means ± SE for 12 (VI) or 10 (DRL) rats expressed as a percentage of control performance. Control responses per minute (mean ± SD) under the VI schedule = 12.5 ± 12.1. Control responses and reinforcers per hour under the DRL schedule = 70 ± 7 and 11 ± 2, respectively. *Significantly different from control performance, $p < 0.05$; $p < 0.01$.

Effects of the PDE3-Selective Inhibitor Milrinone

At the dose range tested, milrinone did not significantly alter response or reinforcement rates of rats under the DRL 72-s schedule, $F(5, 25) = 1.42$ and 1.58, for response and reinforcement rate, respectively (Fig 3). At a dose of 3 mg/kg, milrinone tended to increase response rate and decrease reinforcement rate.

Milrinone did not significantly affect response rate under the VI 30-s schedule, $F(5, 50) = 1.82$ (Fig. 3). At the 3-mg/kg dose, milrinone tended to increase the mean response rate to 175% of the control rate.

Effects of the PDE4-Selective Inhibitor Rolipram

Rolipram altered response and reinforcement rates under the DRL 72-s schedule in a dose-dependent manner, $F(4, 36) = 98.73$ and 10.29, for response and reinforcement rate, respectively; $p < 0.01$ (Fig. 4); at the 0.1-mg/kg dose, mean response rate was reduced to 55% of control and mean reinforcement rate was increased to 155% of control. When administered at a dose of 0.3 mg/kg, rolipram produced large reductions in both mean response and reinforcement rates.

Rolipram produced biphasic effects on behavior maintained under the VI 30-s schedule, $F(5, 50) = 23.56$, $p < 0.01$ (Fig. 4). Administration of doses of 0.001 to 0.01 tended to increase response rate; a significant increase in response rate was observed at a dose of 0.003 mg/kg. Administration of 0.1 mg/kg reduced mean response rate under the VI schedule to 6% of the control response rate.

Effects of the PDES-Selective Inhibitor Zaprinast

Zaprinast produced dose-dependent effects on DRL behavior, $F(5, 45) = 7.41$ and 2.74, $p < 0.01$ and $p < 0.05$, for response and reinforcement rate, respectively (Fig. 5). At doses of 0.3 to 3 mg/kg, zaprinast did not affect mean response or reinforcement rate. However, when administered at a dose of 10

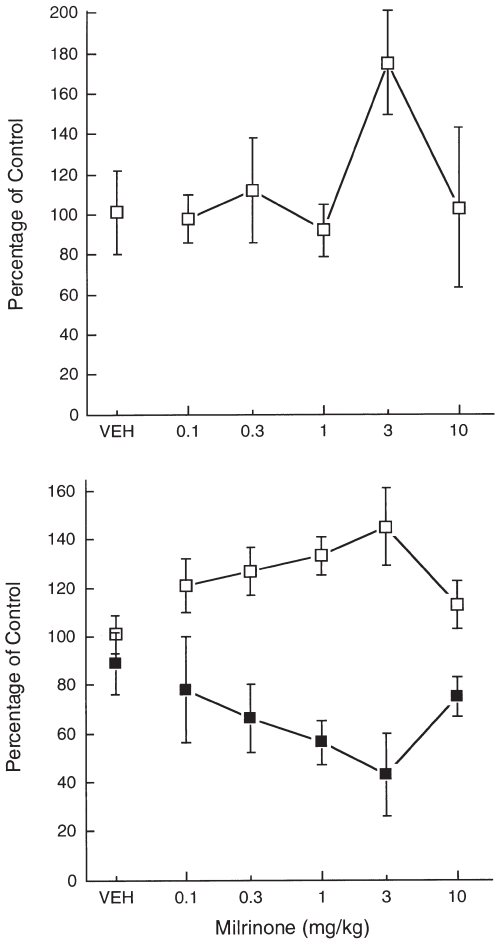

FIG. 3. The effects of the PDE3-selective inhibitor milrinone on response rate of rats under a VI 30-s schedule (upper panel) or on response rate (open squares) and reinforcement rate (filled squares) of rats under a DRL 72-s schedule (lower panel). Points represent the means ± SE for 11 (VI) or 6 (DRL) rats expressed as a percentage of control performance. Control responses per minute (mean ± SD) under the VI schedule = 19.8 ± 10.1. Control responses and reinforcers per hour under the DRL schedule = 71 ± 17 and 12 ± 6, respectively.

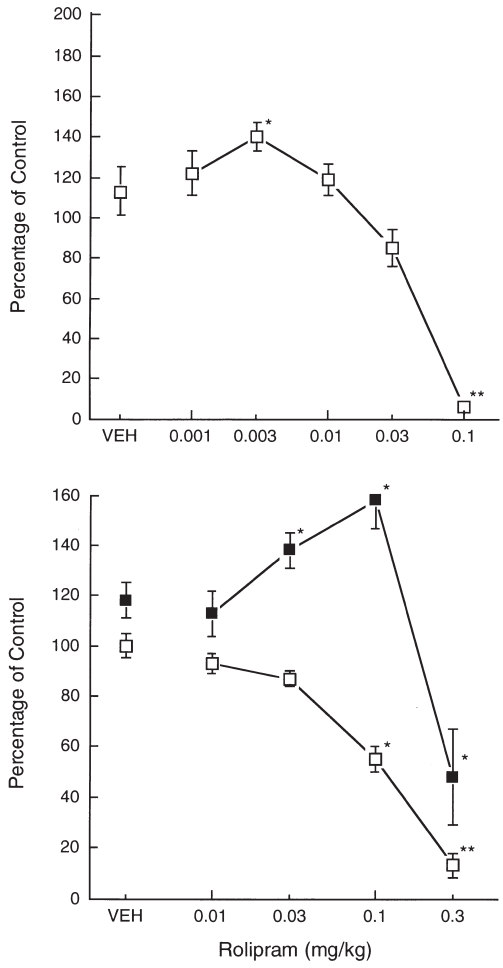

FIG. 4. The effects of the PDE4-selective inhibitor rolipram on response rate of rats under a VI 30-s schedule (upper panel) or on response rate (open squares) and reinforcement rate (filled squares) of rats under a DRL 72-s schedule (lower panel). Points represent the mean ± SE for 11 (VI) or 10 (DRL) rats expressed as a percentage of control performance. Control responses per minute (mean ± SD) under the VI schedule = 23.5 ± 22.2. Control responses and reinforcers per hour under the DRL schedule = 68 ± 9 and 12 ± 3, respectively. *Significantly different from control performance, $p < 0.05$; **$p < 0.01$.

mg/kg, zaprinast reduced response rate and increased reinforcement rate.

Zaprinast reduced response rate under the VI 30-s schedule in a dose-dependent manner, F (5, 50) = 7.90, $p < 0.01$ (Fig. 5). At the lower doses, 0.3 to 3 mg/kg, zaprinast did not affect mean response rate. However, when administered at doses of 10 and 30 mg/kg, zaprinast reduced mean response rate to 52 and 18% of control, respectively.

Effects of the Tricyclic Antidepressant Desipramine

Desipramine produced dose-dependent reductions in response rate under the DRL 72-s schedule, $F(5, 45) = 17.48, p < 0.01$ (Fig. 6); at doses of 3 and 10 mg/kg, these were accompanied by increases in mean reinforcement rate, $F(5, 45) = 4.99$, $p < 0.01$. The maximum increase in reinforcement rate, to 148% of control, was observed following administration of 10 mg/kg desipramine.

Desipramine produced biphasic effects on response rate under the VI 30-s schedule, $F(8, 72) = 13.55, p < 0.01$ (Fig. 6). At a dose of 0.03 mg/kg, desipramine increased response rate to 166% of control. At all other doses tested, desipramine either reduced mean response rate or had no effect. At doses of 0.3 to 18 mg/kg, desipramine-induced reductions in response rate to 71 to 14% of the control rate were observed.

DISCUSSION

The PDE4-selective inhibitor rolipram decreased response rate and increased reinforcement rate under a DRL 72-s schedule. This confirms an earlier report showing that rolipram, as well as three other inhibitors of PDE4, produce antidepressant-like effects on DRL behavior (24). In the earlier study, maximum increases in mean reinforcement rate to more than 200% of control were observed. By contrast, roli-

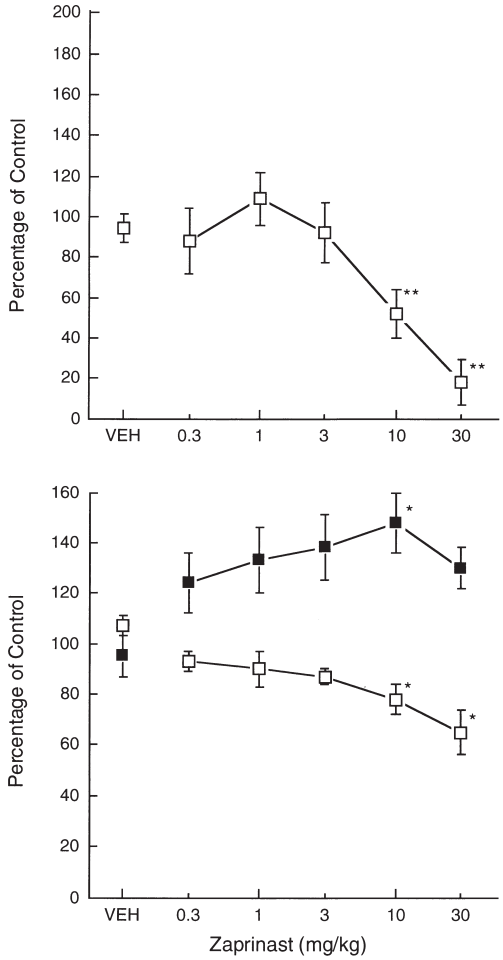

FIG. 5. The effects of the PDE5-selective inhibitor zaprinast on response rate of rats under a VI 30-s schedule (upper panel) or on response rate (open squares) and reinforcement rate (filled squares) of rats under a DRL 72-s schedule (lower panel). Points represent the means ± SE for 11 (VI) or 10 (DRL) rats expressed as a percentage of control performance. Control responses per minute (mean ± SD) under the VI schedule = 16.3 ± 11.8. Control responses and reinforcers per hour under the DRL schedule = 69 ± 10 and 12 ± 3, respectively. *Significantly different from control performance, $p < 0.05$.

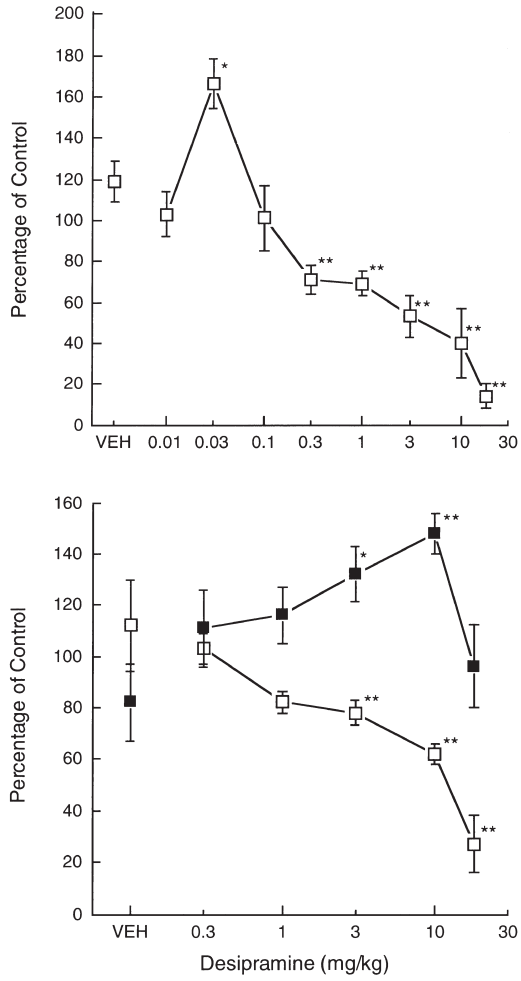

FIG. 6. The effects of the tricyclic antidepressant desipramine on response rate of rats under a VI 30-s schedule (upper panel) or on response rate (open squares) and reinforcement rate (filled squares) of rats under a DRL 72-s schedule (lower panel). Points represent the means ± SE for ten (VI) or ten (DRL) rats expressed as a percentage of control performance. Control responses per minute (mean ± SD) under the VI schedule ± 12.8 ± 11.8. Control responses and reinforcers per hour under the DRL schedule = 66 ± 10 and 15 ± 4, respectively. *Significantly different from control performance, $p < 0.05$; **$p < 0.01$.

pram only increased reinforcement rate to about 150% of control in the present study. However, the effect of rolipram was quantitatively similar to the effect of the tricyclic antidepressant desipramine, which increased mean reinforcement rate to 148% of control. It is likely that differences in control response and reinforcement rate may have contributed to the somewhat reduced effect of rolipram in the present study compared to the earlier report. In the present study, mean control response and reinforcement rates were 71 responses per hour and 12.2 reinforcers per hour, respectively. In the previous study, mean control response and reinforcement rates were 102 responses per hour and 7.5 reinforcers per hour, respectively. Differences in control response and reinforcement rates can result in pronounced differences in the effects of drugs on behavior maintained under a DRL 72-s schedule (27).

In addition to affecting DRL behavior, rolipram produced biphasic effects on behavior maintained under the VI 30-s schedule. At low doses (0.001 to 0.01 mg/kg), rolipram tended to increase response rate. A similar increase in VI response rate occurred after administration of 0.03 mg/kg desipramine. This rate-increasing effect of desipramine is somewhat unusual in rats (39); rate increases have been reported in pigeons under fixed-interval schedules (5,43). In contrast to its effects on VI behavior, at no dose tested did rolipram or desipramine increase response rate under the DRL schedule; tests of doses lower than those currently examined revealed no evidence for a rate-increasing effect of these drugs on DRL behavior (unpublished observations). At a dose of 0.1 mg/kg, rolipram reduced mean response rate to near zero under the VI schedule. By contrast, at this dose, response rate under the DRL schedule was reduced to 55% of control, suggesting that VI behavior exhibited somewhat greater sensitivity to rolipram than did DRL behavior.

Among the other drugs tested, only the PDE5-selective inhibitor zaprinast produced a pattern of behavioral effects similar to those of rolipram and desipramine. Zaprinast, at doses of 10 and 30 mg/kg, reduced response rate and increased reinforcement rate under the DRL schedule. However, in contrast to the biphasic effects of rolipram on VI behavior, at the dose range tested, administration of zaprinast only reduced response rate. While zaprinast is a relatively selective inhibitor of PDE5 (18), it is unclear whether the behavioral effects observed are due to inhibition of this isozyme. A number of other effects of zaprinast thought to be mediated by PDE5 are reported to occur at lower doses than those that affected DRL behavior in the present study (6,48). In a study directly comparing the effects of rolipram and zaprinast in monkeys, Howell (15) reported little effect of zaprinast on respiration and fixed-interval behavior at doses up to 5.6 mg/kg in monkeys. By contrast, effects were observed with rolipram at doses as low as 0.1 mg/kg. It remains to be determined whether the antidepressant-like effect of zaprinast on DRL behavior represents a PDE5-mediated effect or results from an action of high doses of this drug on one or more PDE4 subtype.

The PDE1-selective inhibitor vinpocetine, the PDE2-selective inhibitor trequinsin, and the PDE3-selective inhibitor milrinone did not produce antidepressant-like effects on DRL behavior. At the dose range tested, vinpocetine did not affect either VI or DRL behavior, even though this dose range has been shown to be behaviorally relevant in studies utilizing passive avoidance behavior (2,28). Milrinone actually tended to increase response rate and reduce reinforcement rate at some doses. Although trequinsin did reduce response rate under the DRL schedule, these reductions were not accompanied by increases in reinforcement rate; similar disruptions of DRL behavior have been observed for other drugs with sedative effects (40). Little is known of the behavioral consequences of inhibition of PDE2 or PDE3. However, at the dose ranges tested, inhibitors of these PDE families have been show to alter cardiovascular measures (17,20).

The antidepressant-like behavioral effect of rolipram on DRL behavior is consistent with reports of the effects of this drug, as well as other PDE4-selective inhibitors, on other preclinical tests sensitive to antidepressant drugs. In particular, rolipram and related drugs have been shown to reduce the time of immobility in a forced-swim test and to reverse behavioral changes produced by olfactory bulb lesions (19,31,34). In general, it appears that these effects are related to inhibition of PDE4 (24); however, the present results raise that possibility that inhibition of PDE5 may produce similar, antidepressant-like behavioral effects. Further, other pharmacological effects of rolipram, such as inhibition of adenosine uptake (32), may contribute to some of its behavioral effects, particularly the sedative effects observed at higher doses (12).

To date, 16 PDE4 variants have been identified, and it is likely that additional ones exist. The variants are the result of different initiation sites and splice variants of four distinct genes (i.e., PDE4A, PDE4B, PDE4C, and PDE4D). Of these four main PDE4 subtypes, all but PDE4C have been shown to be expressed in high amounts in the brain (8,45). It is possible that the antidepressant-like behavioral effects may result predominantly from inhibition of a particular PDE4 variant. It has been found that two PDE4A variants, PDE4A5 and a presently unidentified PDE4A variant, are associated with beta adrenergic receptor-linked adenylyl cyclase in rat cerebral cortex and hippocampus (57). Given the importance of this receptor–effector system in the mediation of the effects of antidepressant drugs (10,26,29), it would be of interest to begin to investigate the role of PDE4A variants in the brain in the mediation of antidepressant like behavioral effects.

ACKNOWLEDGEMENTS

The authors thank Schering AG (Berlin, Germany) and Hoechst Celanese (Somerville, NJ) for their generous gifts of rolipram and trequinsin, respectively. This study was supported by research grants and an Independent Scientist Award from the National Institute for Mental Health.

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