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Antidepressant Effects of Rolipram in a Genetic Animal Model of Depression: Cholinergic Supersensitivity and Weight Gain

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OVERSTREET, D. H., K. DOUBLE AND G. D. SCHILLER. *Antidepressant effects of rolipram in a genetic animal model of depression: Cholinergic supersensitivity and weight gain.* PHARMACOL BIOCHEM BEHAV **34**(4) 691–696, 1989.—The effects of rolipram, a new generation antidepressant which is a selective inhibitor of phosphodiesterase, on the selectively bred Flinders Sensitive Line (FSL) of rats, a genetic animal model of depression, was studied. Acutely, rolipram produced comparable decreases in temperature and activity in the FSL and the Flinders Resistant Line (FRL) rats. Upon chronic treatment there was a trend for rolipram to counteract the shock-induced suppression of activity in the FSL rats, suggesting an antidepressant-like effect. However, both groups gained a significant amount of weight, which appeared to be associated with polydipsia and polyuria. In addition, both groups were significantly more affected by the muscarinic agonist, oxotremorine, than their vehicle-treated counterparts. Thus, the FSL rats, which are genetically supersensitive to cholinergic agonists, are even more sensitive following chronic treatment with rolipram. These unexpected findings suggest that rolipram may not be appropriate as an antidepressant for humans because of undesirable side effects.

Cholinergic supersensitivity FSL rats Animal model of depression Weight gain Polydipsia

ROLIPRAM, a selective inhibitor of phosphodiesterase, counteracts reserpine-induced hypothermia in mice (19), as do traditional antidepressants. However, rolipram is able to reverse reserpine-induced hypothermia even in monoamine-depleted mice, indicating a postsynaptic action (20). Therefore, rolipram, unlike the traditional antidepressants which rely on intact monoaminergic synaptic terminals for their actions, can "stimulate both the presynaptic as well as the postsynaptic components of the monoaminergic transmission" (20).

Recently it has been demonstrated that rolipram has little or no antimuscarinic effects in animals (21). In fact, upon acute administration, rolipram produces hypokinesia and hypothermia, which have been more commonly associated with the actions of muscarinic agonists (8,18). Rolipram thus has both antidepressant potential and virtually no antimuscarinic side effects; it could become a useful antidepressant, particularly in the elderly who often cannot tolerate the antimuscarinic side effects of traditional antidepressants (7,16).

Recently we have developed a line of rats which has a genetically related increased cholinergic (muscarinic) sensitivity. These Flinders Sensitive Line (FSL) rats are more sensitive to muscarinic agonists, as are humans with a tendency towards depression, and have other features in common with depressed humans (12,13). Because cholinergic neurons interact with muscarinic receptors coupled with phosphatidyl inositol (PI) (10), it is likely that the FSL rats have a greater generation of PI. Furthermore, because rolipram inhibits phosphodiesterase, thereby elevating cyclic AMP, another second messenger, it is possible that chronic treatment with rolipram may counteract the cholinergic overactivity in the FSL rats. The present study was designed to examine this hypothesis. While rolipram did in fact reduce the "depressive" tendencies of the FSL rats, it also produced several unexpected side effects.

METHOD

Animals

The animals were females from the selectively bred FSL and FRL rats (Sprague-Dawley derived). The rats came from the 36th generation; they weighed 250–320 g and were approximately 120 days old at the start of the experiment. They were housed in polypropylene cages in groups of 8 under conditions of continuous (24 hr) illumination and with free access to food and water. During

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the latter stages of rolipram treatment, the rats were housed in individual cages so that water intake could be assessed (see below).

Drugs

Rolipram [4-(3-cyclopentyloly-4-methoxyphenyl)-2-pyrro-lidone] was a gift from Schering, West Berlin. It was suspended in isotonic saline containing 5% w/v dimethyl sulfoxide (DMSO). Oxotremorine sesquifumarate, atropine methyl nitrate and salbutamol hydrochloride were purchased from Sigma (St. Louis). They were dissolved in isotonic saline. All drugs were injected subcutaneously in volumes of 1 ml/kg.

Apparatus

General activity was recorded in Perspex open field chambers (60 × 30 cm) by measuring the number of lines crossed in a specified time period (usually one minute). Footshock was delivered by placing the rats in a Y-maze with a grid floor and switching on a 1 mA current from a shocker (Flinders School of Biological Sciences workshop) for 2 sec. Core body temperature was recorded by inserting a YSI temperature probe 6 to 8 cm into the rectum and reading the output on an Accurex 9001C Thermistor Thermometer.

Procedure

After initial handling, the animal's baseline open field activities were determined. Preliminary studies were then conducted to discover a suitable dose of rolipram and an appropriate route of administration. It was found that a subcutaneous injection of 2.7 mg/kg rolipram produced equivalent acute effects on activity and body temperature as an intraperitoneal injection.

Rats (FSL and FRL) were randomly divided into two groups. One group received chronic daily injections of rolipram; the other group received chronic daily injections of the DMSO-saline vehicle. It was noted during this period that the rolipram-treated rats were gaining weight and the sawdust in their cages was wet. Rats were then individually housed for a period of four days (between 13–21 days after chronic treatment began). Water consumption was monitored by weighing water bottles on a daily basis. Water bottles left on empty cages were used to estimate drip rates; values reported here are corrected for drip rates. The rat's intake was taken as the average of the four days' values.

On Day 13 of the chronic treatment period the rats were taken and individually placed in the Y-maze to receive a footshock. Immediately after receiving the footshock, each rat was placed in the open field for a one-minute recording of activity. Footshock-induced suppression of activity was studied by noting the changes in activity from the rats' previously recorded baselines.

On Day 14 of the chronic treatment period the rats were "challenged" with oxotremorine, a muscarinic agonist. An injection of 2 mg/kg methyl atropine was followed by a 0.15 mg/kg injection of oxotremorine 15 minutes later. Core body temperature was then recorded 30 minutes after the injection of oxotremorine. These treatments preceded the normal daily injections of rolipram and vehicle, so they were given approximately 24 hours after the last chronic injection.

On Day 21 of the chronic treatment period the rats were "challenged" with salbutamol, a beta adrenergic agonist. Open field activity was recorded 15 minutes and core body temperature 30 minutes after the injection of 1 mg/kg salbutamol.

Two days later (24 hours after the last chronic injection) the rats were sacrificed by decapitation and the cerebral cortex, striatum and hippocampus were dissected out and homogenized in phosphate buffer (0.05 M, pH 7.4). Within several weeks muscarinic receptor binding assays were conducted, using ^{3}H-QNB as the radioactive ligand. Atropine sulfate was added in excess to certain tubes to provide a measure of nonspecific binding. Approximately 100 μg of tissue were incubated with the QNB (in a final volume of 2 ml) at 25°C for 90 min. The samples were then filtered through Whatman GF/B filters using a Brandel receptor binding Harvestor (m-24R). The samples were counted at 45% efficiency in a Beckman LS-5800 Scintillation Counter (in 3 ml Beckman Ready Value Scintillation Fluid). Protein was determined by the Lowry method with BSA as standard. Saturation binding parameters were determined using nonlinear regression least squares fit to all data with "Curfit-3" (Program courtesy of Dr. Terrell Gibbs, SUNY, Brooklyn, NY).

Assays of beta adrenoceptors were not carried out because their decrease after chronic rolipram treatment has been previously reported (17).

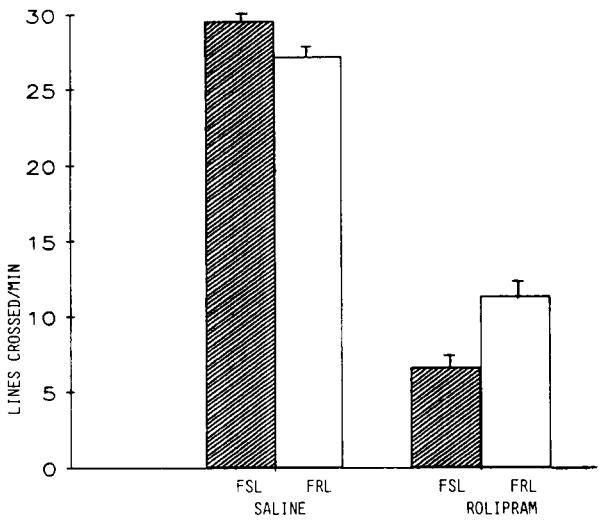

FIG. 1. The effects of acute rolipram on locomotor activity in FSL and FRL rats. A one-minute sample of activity in a rectangular open field was recorded 30 minutes after subcutaneous injection of 2.7 mg/kg rolipram or vehicle.

Data Analysis

For the majority of the data two-way analyses of variance were carried out. The line of rat and the type of treatment were the two main effects. Thus, there were four treatment groups: FSL treated with vehicle; FSL treated with rolipram; FRL treated with vehicle; FRL treated with rolipram. On some occasions, followup Student's *t*-tests were used to clarify the findings.

RESULTS

The mean baseline activities of the four groups were not significantly different from each other, varying from a high of 32.1 line crossings to a low of 27.3. Similarly, their baseline core body temperatures were not different (data not shown).

As can be seen in Fig. 1, acute rolipram had a behaviorally depressant effect which was comparable in the two lines. Analysis of these data indicated that there was a significant treatment effect only, $F(1,27) = 32.257$, $p < 0.001$; neither the line nor the interaction effects were significant. Rolipram also had an acute hypo-

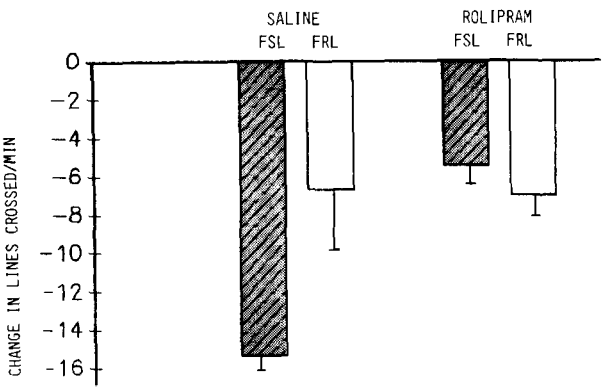

FIG. 2. The effect of footshock on activity in FSL and FRL rats chronically treated with rolipram or vehicle. A one-minute sample of activity was recorded immediately after exposure to a 2-second, 1 mA footshock and 24 hours after a series of 12 daily 2.7 mg/kg SC injections of rolipram or vehicle. Activity is expressed as a change from a previously established baseline.

thermic effect, which was comparable in the two lines (data not shown).

Following exposure to 2 seconds footshock, FSL rats treated with vehicle exhibited a large decline in activity, as can be seen in Fig. 2. The other three groups had smaller, approximately equal decreases in activity. However, there was a very large variability in some groups, so the two-way ANOVA did not reveal any statistically significant line or treatment differences. On subsequent analysis, the FSL rats treated with rolipram were significantly more active (i.e., less inhibited by shock) than the FSL rats treated with vehicle ($t=2.49$, $p<0.05$). So there is some suggestion that rolipram has an "antidepressant" effect in the FSL rats.

Figure 3 summarizes the changes in temperature induced by the muscarinic agonist, oxotremorine. It can be readily seen that the FSL rats have greater drops in temperature than their FRL counterparts; likewise, the rolipram-treated rats were more affected than their vehicle-treated counterparts. The two-way ANOVA completely supported these visual impressions: Both the line, $F(1,26)=11.59$, $p<0.01$, and the treatment, $F(1,26)=16.57$, $p<0.001$, effects were highly significant, but the interaction effect was not. Thus, rolipram makes the FSL rats, which are already supersensitive to cholinergic agonists, even more sensitive.

Figure 4 summarizes the changes in temperature induced by the beta adrenergic agonist, salbutamol. There was a strong trend for subsensitivity to salbutamol in the rolipram-treated rats and a smaller indication of the FSL being less affected. The two-way ANOVA confirmed that the treatment effect was highly significant, $F(1,27)=24.42$, $p<0.001$, while the line effect was not, $F(1,27)=3.41$, $p<0.08$. A similar trend for the rolipram-treated rats in each line to exhibit smaller decreases in activity was seen, but the two-way ANOVA revealed no significant main effects (data not shown).

The change in weight shown by the vehicle- and rolipram-treated rats is illustrated in Fig. 5. The rolipram-treated rats gained significantly more weight than the vehicle-treated rats, $F(1,27)=26.14$, $p<0.001$. The FRL rats gained significantly more weight (20–30 g) than the FSL rats, $F(1,27)=7.07$, $p<0.01$. The tendency for the rolipram-treated FRL rats to gain more weight than their FSL counterparts was associated with a nearly significant interaction effect in the two-way ANOVA, $F(1,27)=4.09$, $p<0.053$.

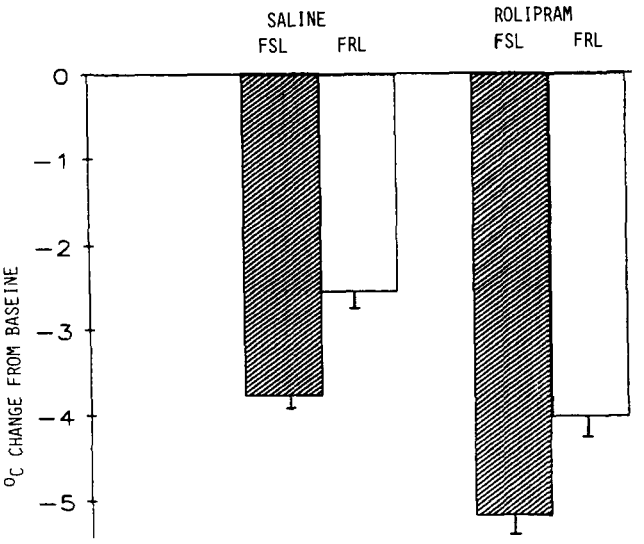

FIG. 3. The effect of oxotremorine on temperature in FSL and FRL rats chronically treated with rolipram or vehicle. Temperature was recorded 45 and 30 minutes after the acute injections of methyl atropine nitrate (2 mg/kg) and oxotremorine sesquifumarate (0.15 mg/kg), respectively, and 24 hours after a series of 13 daily 2.7 mg/kg injections of rolipram or vehicle. Temperature is expressed as a change from a previously established baseline.

The increase in body weight shown by the rolipram-treated rats was associated with an increase in water consumption, as illustrated in Fig. 6. However, the greater increase in weight in the FRL than the FSL rats did not correspond to a greater water consumption. The two-way ANOVA revealed a statistically significant treatment effect, $F(1,27)=7.32$, $p<0.01$, but no significant line effect ($p<0.1$). There was a nonsignificant trend for the rolipram-treated FRL rats to be more affected than their FSL counterparts (interaction $p<0.09$). It was also observed that rolipram-treated rats had wetter sawdust during cleaning of their cages, but no attempt was made to quantify this.

Initially, saturation assays of striatal tissue from three rats

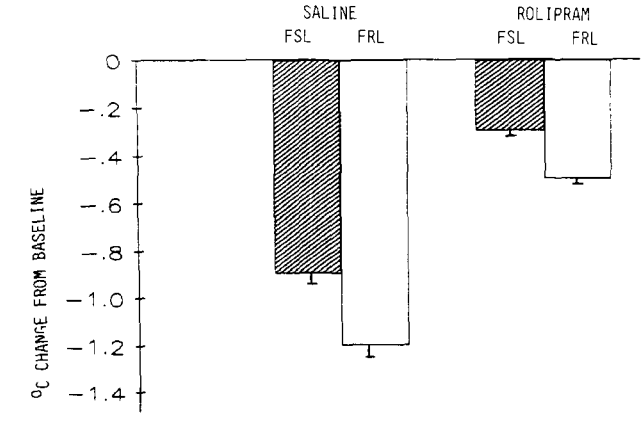

FIG. 4. The effect of salbutamol on temperature in FSL and FRL rats chronically treated with rolipram or vehicle. Temperature was recorded 30 minutes after the acute injection of 1 mg/kg salbutamol hydrochloride and 24 hours after a series of 20 daily 2.7 mg/kg injections of rolipram or vehicle. Data represent changes from previous baselines.

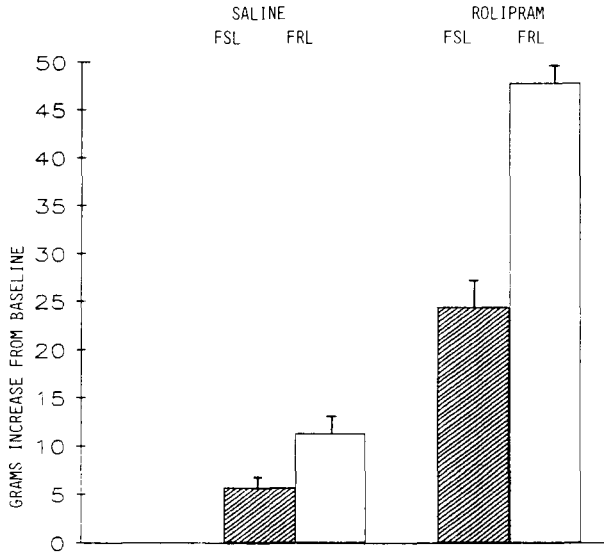

FIG. 5. Weight change in FSL and FRL rats during 10 days of chronic treatment with rolipram (2.7 mg/kg) or vehicle.

from each of the four groups were carried out. Analyses of these data with a nonlinear regression program indicated that there were no differences in the K_d's (see Table 1). There was, however, a substantial decrease in B_{max} in the rolipram-treated rats (Table 1). Preliminary studies in which brain tissue was preincubated with 5×10^{-5} M rolipram did not reveal any effect on QNB binding. So the decrease seen in the rats chronically treated with rolipram is unlikely to be due to the in vitro effects of rolipram.

Because of the lack of effect of line or treatment on K_d, the tissues from the hippocampus and cerebral cortex were incubated with a single, near saturating concentration of QNB (0.8 nM). These results also revealed similar binding values for the FSL and

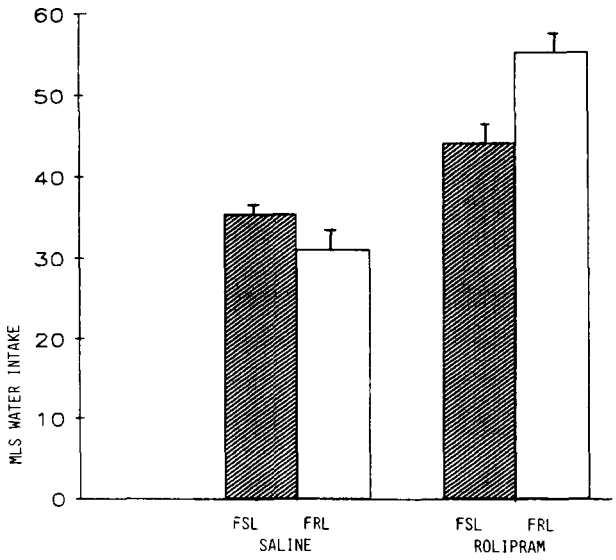

FIG. 6. Water intake in FSL and FRL rats during chronic treatment with rolipram or vehicle. Each rat's intake was individually measured over a 4-day period and an average was taken. The data represent the means of these averages for 6–8 rats.

TABLE 1

EFFECTS OF CHRONIC ROLIPRAM TREATMENT ON STRIATAL MUSCARINIC RECEPTOR AFFINITY AND DENSITY IN FSL AND FRL RATS

Group	N	Affinity (K_d)	Density (B_{max})
FSL-vehicle	3	41.9 ± 4.0	1.82 ± 0.49
FSL-rolipram	3	49.5 ± 8.5	1.40 ± 0.17 (23%)
FRL-vehicle	3	49.8 ± 6.1	2.03 ± 0.41
FRL-rolipram	3	45.1 ± 1.9	1.25 ± 0.05 (38%)

FRL rats, so the data were combined to permit a comparison of the effect of rolipram in a larger sample. For both tissues the binding was higher in the vehicle-treated rats (0.99 ± 0.05 pmol/mg protein for cerebral cortex and 0.78 ± 0.02 for hippocampus) than in the rolipram-treated rats (0.94 ± 0.03 and 0.71 ± 0.02, respectively). Thus, a similar trend to that observed for the striatum was obtained, but the differences were not statistically significant.

DISCUSSION

Rolipram has been shown in several recent studies to be as effective an antidepressant as the tricyclics (1, 7, 22), and is thought to be potentially very useful in the elderly because of its absence of antimuscarinic side-effects (16,21). The results of the present study provide qualified support for the antidepressant potential of rolipram (Fig. 2), but raise a number of questions/problems about the chronic use of rolipram. These have not been addressed in any previously published paper, so it is not possible to determine the extent of these problems. Nevertheless, these problems should be carefully considered before unqualified support is given to rolipram's therapeutic potential in depressive disorders.

Cholinergic Supersensitivity

The increased sensitivity of the FSL rats to oxotremorine (Fig. 3) is consistent with previous work with the FSL and FRL rats (12, 13, 15). However, there was no corresponding increase in muscarinic receptor binding in brain tissues from these rats (Table 1), as has been demonstrated in male rats (14,15). These findings suggest that mechanisms other than increased receptors also contribute to the cholinergic supersensitivity observed in FSL rats.

The dramatic increase in sensitivity to oxotremorine exhibited by the rolipram-treated FSL and FRL rats was not anticipated (Fig. 3), nor was the absence of a parallel increase in muscarinic receptor binding. If anything, there was a decrease in muscarinic receptors in the rolipram-treated rats (Table 1). These findings provide further evidence for a dissociation between functional receptor sensitivity and receptor concentration, an increasingly common finding in studies of receptor plasticity. The increased sensitivity to muscarinic agonists in rats chronically treated with rolipram has also been commonly observed following chronic treatment with classic antidepressants (13), but such findings would be expected because of their well documented antimuscarinic effects. Why rolipram, which has essentially no antimuscarinic effects (16,21), should also lead to increased muscarinic sensitivity is not immediately clear.

Rolipram has been reported to be a selective inhibitor of cyclic adenosine 3′,5′-monophosphate phosphodiesterase and to have, therefore, both pre- and postsynaptic effects on noradrenergic transmission (17,20). The subsensitivity to the beta adrenergic agonist salbutamol observed in our study (Fig. 4) is consistent with

an earlier report of reduced concentration of beta adrenoceptors in rolipram-treated rats (19). Such changes may provide a clue to the muscarinic supersensitivity observed in rolipram-treated rats. There have been several instances reported where treatments which alter the tone (sensitivity/state) of one neurotransmitter system have resulted in secondary alterations in another. For example, rats which have been chronically treated with the dopamine antagonist haloperidol are subsensitive to muscarinic agonists as well as supersensitive to dopamine agonists (6). The FSL rats exhibit changes in sensitivity to a range of other neurotransmitter specific agents (13). Thus, the rolipram-induced cholinergic supersensitivity may be secondary to a beta adrenoceptor subsensitivity. Dilsaver (2) has made additional suggestions as to how antidepressants without antimuscarinic actions might induce cholinergic supersensitivity when given chronically.

Whatever the underlying mechanism, muscarinic supersensitivity clearly accompanies chronic treatment with rolipram. This observation is cause for concern because it suggests that there may be a rebound depression when rolipram is withdrawn. Such a phenomenon has been observed following cessation of treatment with classical antidepressants (3) and cholinergic mechanisms have been implicated (4). To date, the studies on rolipram's effects in humans have not explored this possibility (1, 7, 22); however, it has been recently reported that withdrawal from trazodone, another new generation antidepressant without antimuscarinic effects, may lead to rebound withdrawal effects which are sensitive to atropine (11). Certainly, similar information for rolipram is necessary before approval is given for its routine use in humans.

Weight Gain

The second unexpected finding in this study was the substantial weight gain in the rolipram-treated rats (Fig. 5). Again, no mention has been made as to whether this has been a problem in the human studies (1, 7, 16, 22). Weight gains are widely recognized as problems with antidepressants and lithium (5). The mechanisms underlying these weight gains have not been systematically studied, but factors such as increased appetite and polydipsia have been implicated. There was evidence for increased water intake and polyuria in the rolipram-treated rats (Fig. 6), but these were not followed up. Such findings, even though in rats, suggest that further studies of human subjects should involve a more careful examination of the possibility of weight gain and polydipsia. Nevertheless, the detection of these side effects in humans may not preclude the use of rolipram in the treatment of depression because many other useful antidepressant drugs have similar side effects (5). It would still be helpful to know that a potential for such side effects exists before rolipram is approved for widespread use in humans.

Conclusions

The trend for rolipram to reduce the effects of shock in FSL rats supports previous animal and human research demonstrating that rolipram may be a useful antidepressant. However, chronic rolipram treatment was also associated with cholinergic supersensitivity and weight gain, side effects which, if observed in humans, might limit its usefulness as an antidepressant. Further human studies are warranted before rolipram should be considered for routine use as an an antidepressant.

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REFERENCES

1. Bertolino, A.; Crippa, D.; diDio, S.; Fichte, K.; Musmeci, G.; Porro, V.; Rapisarda, V.; Sastre-y-Hernandez, M.; Schratzer, M. Rolipram versus imipramine in inpatients with major, "minor" or atypical depressive disorder: A double blind double-dummy study aimed at testing a novel therapeutic approach. Int. Clin. Psychopharmacol. 3:245–254; 1988.
2. Dilsaver, S. C. Pathophysiologies of substance abuse and affective disorders: an integrative model. J. Clin. Psychopharmacol. 7:1–10; 1987.
3. Dilsaver, S. C.; Greden, J. F. Antidepressant withdrawal phenomena. Biol. Psychiatry 19:227–256; 1984.
4. Dilsaver, S. C.; Greden, J. F. Antidepressant withdrawal-induced activation (hypomania and mania): Mechanisms and theoretical significance. Brain Res. Rev. 7:29–84; 1984.
5. Garland, E. J.; Remick, R. A.; Zis, A. P. Weight gain with antidepressants and lithium. J. Clin. Psychopharmacol. 8:323–330; 1988.
6. Gianutsos, G.; Lal, H. Alteration in the action of cholinergic and anticholinergic drugs after chronic haloperidol: Indirect evidence for cholinergic hyposensitivity. Life Sci. 18:515–520; 1976.
7. Horowski, R.; Sastre-y-Hernandez, M. Clinical effects of the neurotropic-selective cAMP phosphodiesterase inhibitor rolipram in depressed patients: Global evaluation of preliminary reports. Current Ther. Res. 38:23–29; 1985.
8. Janowsky, D. S.; El-Yousef, M. K.; Davis, J. M.; Sekerke, H. J. A cholinergic-adrenergic hypothesis of mania and depression. Lancet 2:632–635; 1972.
9. Karczmar, A. G. The present and future of the development of anti-OP drugs. Fundam. Appl. Pharmacol. 5:S270–S279; 1985.
10. McKinney, M.; Richelson, E. The coupling of muscarinic receptor to responses. Annu. Rev. Pharmacol. Toxicol. 24:121–146; 1984.
11. Montabelli, D. J.; Zis, A. P. Cholinergic rebound following trazodone withdrawal? J. Clin. Psychopharmacol. 8:73; 1988.
12. Overstreet, D. H. Selective breeding for increased cholinergic function: Development of a new animal model of depression. Biol. Psychiatry 21:49–48; 1986.
13. Overstreet, D. H.; Russell, R. W.; Crocker, A. D.; Gillin, J. C.; Janowsky, D. S. Genetic and pharmacological models of cholinergic supersensitivity and affective disorders. Experientia 44:465–472; 1988.
14. Overstreet, D. H.; Russell, R. W.; Crocker, A. D.; Schiller, G. D. Selective breeding for differences in cholinergic function: Pre- and post-synaptic mechanisms involved in sensitivity to the anticholinesterase, DFP. Brain Res. 294:227–232; 1984.
15. Pepe, S.; Overstreet, D. H.; Crocker, A. D. Enhanced benzodiazepine responsiveness in rats with increased cholinergic function. Pharmacol. Biochem. Behav. 31:15–20; 1988.
16. Ross, C. E.; Toon, S.; Rowland, M.; Murray, G. H.; Meya, U. A study to assess the anticholinergic activity of rolipram in healthy elderly volunteers. Pharmacopsychiatry 21:222–225; 1988.
17. Schultz, J. F.; Schmidt, G. M. Rolipram, a stereospecific inhibitor of calmodulin-independent phosphodiesterase, causes β-adrenoceptor subsensitivity in rat cerebral cortex. Naunyn Schmeidebergs Arch. Pharmacol. 33:23–30; 1986.
18. Wachtel, H. Characteristic behavioral alterations in rats induced by rolipram and other selective adenosine cyclic 3′,5′-monophosphate phosphodiesterase inhibitors. Psychopharmacology (Berlin) 77:309–316; 1982.
19. Wachtel, H. Potential antidepressant activity of rolipram and other selective cyclic 3′,5′-monophosphate phosphodiesterase inhibitors. Neuropharmacology 22:367–372; 1983.
20. Wachtel, H.; Schneider, H. H. Rolipram, a novel antidepressant drug, reverses the hypothermia and hypokinesia of monoamine-depleted mice by an action beyond postsynaptic monoamine receptors. Neu-

ropharmacology 25:1119–1126; 1986.
21. Wachtel, H.; Loschmann, P. A.; Pietzuch, P. Absence of anticholinergic sensitivity of rolipram, an antidepressant with a novel mechanism of action, in three different animal models in vivo. Pharmacopsychiatry 21:218–221; 1988.
22. Zeller, E.; Stief, B. J.; Pflug, H.; Sastre-y-Hernandez, M. Results of a phase III study of the antidepressant effect of rolipram. Pharmacopsychiatry 17:188–190; 1984.