period following the plateaus corresponds to the silent periods separating the bursts (*1*). The frequency of recurrence of the plateaus and of the bursts also shows parallel behavior. It is thus tempting to speculate that the phasic pattern of firing recorded extracellularly from vasopressin-secreting cells in vivo may be triggered by endogenous plateau potentials. Indeed, such potentials are of appropriate form and magnitude to serve as driver potentials supplying depolarizing current to an axonal impulse-initiating zone (*10*). Nevertheless, in contrast to in vivo experiments where action potentials are recorded from the somata in vitro, we observed no action potentials superimposed on the plateau depolarizations. This is not surprising since action potential generation would be inactivated by a depolarization and fall in the input resistance of the magnitude recorded in our cells. If plateau potentials do exist in vivo, they must be of smaller magnitude to permit somatic impulse generation. The magnitude of the depolarizations recorded in our cultured neurons may be due to the in vitro conditions in which the cells are raised, to their stage of maturation, or to both.

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References and Notes

1. D. A. Poulain and J. B. Wakerley, *Neuroscience* **7**, 773 (1982).
2. P. Legendre, I. Cooke, J. D. Vincent, *J. Neurophysiol.* **48**, 1121 (1982).
3. P. Benda, F. de Vitry, R. Picart, A. Tixier-Vidal, *Exp. Brain Res.* **23**, 29 (1975); see also (*2*).
4. The recordings were performed with KCl (3*M*)-filled glass micropipettes (50 to 70 megohms). A conventional bridge circuit (Dagan 8500) permitted simultaneous voltage recording and injection of current through the same electrode. The preparations, maintained at 30°C, were continuously perfused with balanced salt solution (Hanks), supplemented with 0.6 percent glucose and 2 m*M* Ca^{2+} (final concentration, 4 m*M*), buffered at *p*H 7.2 with 10 m*M* Hepes. Drug applications were made with micropipettes with tip diameters of 8 to 10 μm; the ejections were controlled by a gas pressure system with a micropump (Medical System, BH2).
5. See P. G. Nelson, E. A. Neale, R. L. MacDonald, in *Excitable Cells in Tissue Culture*, P. G. Nelson and M. Liebermann, Eds. (Plenum, New York, 1981), pp. 39–80.
6. W. W. Stewart, *Cell* **14**, 741 (1978); E. A. Neale, R. L. MacDonald, P. G. Nelson, *Brain Res.* **152**, 265 (1978). For more detail on injection of these tracers into the hypothalamic neurons, see also (*2*).
7. M. V. Sofroniew and W. Glasmann, *Neuroscience* **6**, 619 (1981).
8. W. E. Armstrong, J. Scholer, T. H. McNeill, *ibid.* **7**, 679 (1982); I. J. Luqui and C. A. Fox, *J. Comp. Neurol.* **168**, 7 (1976).
9. Immunohistochemical staining was performed directly in the culture dishes. After rinsing in phosphate-buffered saline (PBS, Dulbecco's, Mg^{2+}- and Ca^{2+}-free), the cultures were fixed with 4 percent paraformaldehyde in PBS for 2 hours at room temperature, rinsed well again, and exposed to 0.1 percent Triton X-100 in PBS for 10 minutes. They were then incubated with 1 percent human serum albumen in PBS to prevent nonspecific binding of antibody and immediately incubated with the antibodies, diluted 1:1000 in PBS for 24 hours at 4°C. Certain cultures were then reacted with fluorescein-conjugated sheep antiserum to immunoglobulin G (IgG; Institut Pasteur) at a dilution of 1:10. Other cultures were first reacted with sheep serum against rabbit IgG, diluted 1:30 for 30 minutes, and then with a peroxidase-antiperoxidase complex (Dakopats) diluted 1:75 for 1 hour; peroxidase activity was visualized with diaminobenzidine. Control staining was performed either with rabbit preimmune serum or with the antibody solutions to which the respective peptides were added, free or coupled to Sepharose beads.
10. K. Tazaki and I. M. Cooke, *J. Neurophysiol.* **42**, 1000 (1979).
11. We thank D. Poulain for his help with the manuscript, R. Bonhomme and B. Dupouy for technical assistance, and M. A. Delaage for the sera against vasopressin and oxytocin. Supported by grant DGRST 82 EO 26.

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Differential Effects of Classical and Atypical Antipsychotic Drugs on A9 and A10 Dopamine Neurons

Abstract. *Prolonged treatment with classical antipsychotic drugs decreased the number of spontaneously active dopamine neurons in both the substantia nigra (A9) and the ventral tegmental area (A10) of the rat brain. In contrast, treatment with atypical antipsychotic drugs selectively decreased the number of A10 dopamine neurons. Related drugs lacking antipsychotic efficacy failed to decrease dopamine activity. These findings suggest that the inability of atypical antipsychotic drugs to decrease A9 dopamine neuronal activity may be related to their lower potential for causing tardive dyskinesia and that the inactivation of A10 neurons may be involved in the delayed onset of therapeutic effects during treatment.*

The dopamine (DA) hypothesis of schizophrenia postulates hyperactive DA neurotransmission as an etiological factor in schizophrenic symptomatology (*1*). Despite continued efforts to provide direct support for DA overactivity in unmedicated schizophrenics, the available evidence remains largely uncompelling (*2*). Therefore, the DA hypothesis continues to rest primarily on a pharmacological foundation, which has as its cornerstone the evidence that effective antipsychotic drugs (APD's) dampen DA activity by blocking brain DA receptors (*3*). However, this cornerstone is structurally flawed because DA receptor antagonism is an almost immediate consequence of APD administration whereas antipsychotic efficacy becomes manifest only during prolonged treatment with APD's (*4*). Although this flaw could lead to the eventual collapse of the DA hypothesis, the foundation might be reinforced by demonstrating effects of APD's that develop only as a secondary effect of long-term DA receptor blockade and, therefore, may be causally related to the delayed onset of therapeutic efficacy. However, most reports have suggested that the efficacy of DA receptor antagonism diminishes during treatment with APD's (*5*), a finding that seems incompatible with the slowly developing onset of therapeutic efficacy.

Recently, we confirmed (*6*) the previous finding (*7*) that prolonged haloperidol (HAL) treatment decreases the number of spontaneously active DA-containing neurons in the rat substantia nigra zona compacta (A9) and extended this finding to DA-containing neurons in the ventral tegmental area (A10) (*6*). The nigrostriatal A9 DA system is thought to be involved in APD-induced extrapyramidal side effects such as tardive dyskinesia (TD) (*8*), whereas the mesolimbic and mesocortical A10 DA systems have been implicated in the therapeutic actions of APD's (*1*). Our experiments demonstrated that the decline of spontaneously active DA neurons during HAL treatment is a slowly developing process that occurs earlier and to a greater extent in A10 than in A9. Since the time courses for the decline of A10 and A9 DA activity correspond to the fact that the therapeutic effects of APD treatment precede the onset of TD, we proposed that the inactivation of A10 and A9 DA neurons may be related to the delayed onset of pharmacotherapy and TD, respectively. If this is the case, then it would be expected that "atypical" APD's, which possess a low potential for causing extrapyramidal side effects and TD, would preferentially inactivate A10 DA neurons. This hypothesis was tested in the present experiments by comparing the effects of prolonged treatment with various classical and atypical APD's on the number of spontaneously active A9 and A10 DA neurons as determined using extracellular single-unit recording techniques. We report that atypical APD's differ from classical APD's in that they selectively inactivate A10 DA neurons (*9*).

In these experiments we investigated

the effects of two classical APD's, the butyrophenone HAL and the phenothiazine chlorpromazine (CPZ), and four structurally diverse, atypical APD's: the dibenzodiazepine clozapine (CLZ) and the piperidine side-chain phenothiazine derivative thioridazine (TRZ), which have a relatively low liability for producing TD (*10*), and the substituted benzamide sulpiride and the indole derivative molindone, which, although less well investigated clinically, have also been reported as less likely to cause TD than classical APD's (*11*). For comparison, we studied another substituted benzamide, metoclopramide, because at low doses this DA antagonist exerts only weak antipsychotic effects but can readily induce extrapyramidal side effects and TD (*12*).

Groups of male Sprague-Dawley rats (N = 5 to 6 per group) received 28 daily subcutaneous injections (*13*) of one of the above drugs. Doses were chosen, whenever possible, on the basis of daily clinical doses equivalent to HAL at 0.5 mg/kg (*14*). To ensure that any observed effects were the result of prolonged treatment, experiments in which just one injection was given (N = 4) were also performed. The results from both these groups were compared to those of a control group consisting of rats that received 28 daily subcutaneous injections of drug vehicle (N = 8) and rats that were untreated (N = 4) (*15*). Two hours after the last injection, each rat was anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and mounted in a stereotaxic apparatus. Standard extracellular single-unit recording techniques were used to determine the number of spontaneously active DA neurons in ten descending tracks through both the A9 and A10 regions (*16*).

Similar to the effects produced by HAL (*6*), CPZ significantly affected both A9 and A10 DA neurons, increasing the number of spontaneously active A9 and A10 DA cells per track after single injections while decreasing the number of active DA cells per track in both areas after repeated administration (Fig. 1). In marked contrast to HAL and CPZ, the four atypical APD's produced selective effects on A10 DA cells, increasing the number of A10 DA cells per track after single injections while decreasing the number of active A10 DA cells per track after repeated administration (Fig. 1). Neither form of treatment with any of the atypical APD's significantly affected the number of active A9 DA cells per track. Metoclopramide produced effects that were opposite from those of the four atypical APD's in that only the A9 DA cells were increased and decreased by single and multiple injections, respectively (Fig. 1).

Table 1. Effects of repeated treatment with antipsychotic drugs on the percentage of A9 and A10 dopamine neurons displaying irregular firing patterns.

Drug	Number of rats	A9 (percent cells)	A10 (percent cells)
Control	12	4.2 ± 1.7	6.2 ± 2.7
Haloperidol	6	37.9 ± 8.2*	56.3 ± 9.3*
Chlorpromazine	5	30.6 ± 4.8*	74.8 ± 6.9*
Clozapine	6	6.5 ± 2.9	54.5 ± 8.4*
Thioridazine	5	4.2 ± 2.4	81.8 ± 8.6*
Molindone	5	6.2 ± 3.7	59.2 ± 6.4*
Sulpiride	6	8.8 ± 2.5	44.5 ± 6.2*
Metoclopramide	6	52.2 ± 9.8*	3.3 ± 2.1

*P < 0.01, Dunnett's test.

As previously reported (*6*), coincident with the decline of spontaneously active DA cells per track during prolonged HAL treatment was a significant increase in the percentage of the remaining active DA cells that exhibited discharge patterns uncharacteristic of DA cells recorded in control rats. This irregular firing pattern was characterized primarily by an increase in both the number of short bursting periods and the number of unusually long interspike intervals (*17*). The effect was found to be region-specific in that a significant percentage of irregular-firing DA neurons was encountered only when there was a decreased number of spontaneously active DA cells (Table 1). Thus HAL and CPZ significantly increased the percentage of the remaining active DA cells displaying the irregular pattern in both A9 and A10, the atypical APD's caused a selective increase in the percentage of irregular-firing A10 DA neurons whereas metoclopramide produced this effect only on A9 DA neurons.

To determine whether the decline in the number of spontaneously active DA cells per track and the induction of irregular firing patterns produced by repeated APD administration occurred through depolarization inactivation (*18*), as shown previously in the case of HAL (*6, 7*), the DA agonist apomorphine (0.063 mg/kg), which hyperpolarizes and inhibits its DA cells in untreated rats (*19*), was administered intravenously to rats receiving prolonged APD treatment, and the number of A9 or A10 DA cells (or both) was redetermined in five descending tracks per area. In every case, apomorphine (*APO*) (0.063 mg/kg) significantly reversed the effect. Sample sizes for repeated treatments are as reported in Table 1. Sample size for single-injection experiments was four rats per drug. Abbreviations: haloperidol (*HAL*), chlorpromazine (*CPZ*), clozapine (*CLZ*), thioridazine (*TRZ*), molindone (*MOL*), sulpiride (*SUL*), and metoclopramide (*MET*).

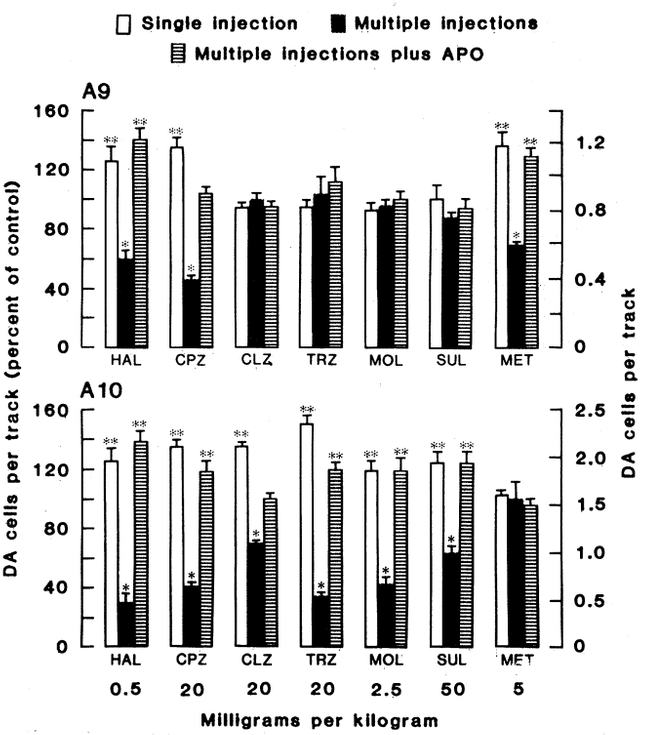

Fig. 1. The effects of short- and long-term treatment with various APD's on the number of spontaneously active A9 and A10 DA cells per track. Values are means ± standard errors. Single asterisks represent values that were significantly below control (P < 0.01) and double asterisks represent values that were significantly above control (P < 0.01). Control values for A9 and A10 were 0.87 ± 0.03 and 1.53 ± 0.04 cells per track, respectively. Note that in every case where repeated treatment with an APD decreased the number of DA cells per track, a single intravenous injection of apomorphine (*APO*) (0.063 mg/kg) significantly reversed the effect. Sample sizes for repeated treatments are as reported in Table 1. Sample size for single-injection experiments was four rats per drug. Abbreviations: haloperidol (*HAL*), chlorpromazine (*CPZ*), clozapine (*CLZ*), thioridazine (*TRZ*), molindone (*MOL*), sulpiride (*SUL*), and metoclopramide (*MET*).

morphine significantly reversed the diminished DA cell count and returned the irregular firing DA cells to a normal firing pattern. These results support the possibility that DA neurons become inactivated due to excessive depolarization during prolonged treatment with APD's (*6, 7, 9*) and that the irregular firing DA neurons may be in a "prequiescent" stage of inactivation (*6*).

To determine whether the decrease in spontaneously active DA neurons and the induction of irregular firing patterns were specific effects of APD's, several other groups of rats ($N = 3$ per group) received 28 daily subcutaneous injections of a variety of compounds lacking antipsychotic efficacy. These control drugs included the antihistaminergic phenothiazine, promethazine HCl (20 mg/kg), the tricyclic antidepressant desipramine HCl (10 mg/kg), the opiate morphine (1 and 15 mg/kg), and the serotonin agonist quipazine (2 mg/kg). Promethazine and desipramine were chosen because of their structural similarity to CPZ. Morphine and quipazine were selected because these drugs can mimic the effects of acute APD's on DA neurons, that is, increase their firing rates and induce a marked depolarization (*20*). After repeated administration, these compounds failed to decrease the number of spontaneously active A9 or A10 DA cells. In fact, repeated treatment with both morphine and desipramine significantly increased the number of active A9 and A10 DA neurons (*21*).

Taken together, these results strongly support our proposal that the time-dependent inactivation of A10 and A9 DA neurons by prolonged administration of APD's may be related to the delayed onset of APD pharmacotherapy and TD, respectively. Thus both classical and atypical APD's significantly decreased the number of spontaneously active A10 DA cells per track. In contrast, only classical APD's, which possess a relatively high potential for inducing TD, decreased the number of spontaneously active A9 DA cells per track. Moreover, low doses of metoclopramide, which do not exert antipsychotic effects but can induce TD (*12*), significantly decreased the number of DA cells per track only in A9. Since single injections of each of the APD's increased the number of spontaneously active DA cells per track only in the areas that were decreased by repeated drug exposure, these short-term effects of APD's could conceivably be used to predict the therapeutic efficacy and extrapyramidal side effect liability of potential APD's. However, the usefulness of such a model would be compromised by the fact that some non-APD's, such as morphine and quipazine, can mimic various effects of single APD injections. In contrast, sustained treatment with these (and other) non-APD's failed to mimic the effects of sustained APD treatment. Thus our results demonstrate that greater precision can be obtained by predicting the clinical effects of potential APD's on the basis of long-term drug administration.

Although the neuronal mechanisms by which classical and atypical APD's exert different pharmacological effects are not known, considerable research has suggested preferential effects of atypical APD's on mesolimbic DA systems (*22*). Previous experiments on the effects of repeated HAL administration on DA neurons have demonstrated that the inactivation of A9 and A10 DA neurons by such treatment can be attenuated by destroying postsynaptic DA receptor–containing cells in the striatum and nucleus accumbens, respectively (*6, 7*). Therefore, this effect of HAL administration appears to be secondary to DA receptor blockade in the forebrain. If the atypical APD's inactivate A10 DA neurons through a similar mechanism, these results suggest important differences between the postsynaptic actions of atypical APD's in limbic and striatal areas and the feedback pathways from these forebrain regions to their respective cells of origin.

In summary, our results suggest that the inability of atypical APD's to inactivate A9 DA neurons may be related to their lower potential for causing TD. The finding that both classical and atypical APD's cause a time-dependent inactivation of A10 DA neurons indicates the importance of the A10 mesolimbic and mesocortical DA systems in mediating the therapeutic actions of APD's and should reinforce the pharmacological foundation supporting the DA hypothesis of schizophrenia.

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References and Notes

1. S. W. Matthysse, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 200 (1973); J. R. Stevens, *Arch. Gen. Psychiatry* **29**, 177 (1973); S. H. Snyder, *Am. J. Psychiatry* **133**, 197 (1976).
2. J. L. Haracz, *Schizophr. Bull.* **8**, 438 (1982).
3. L. L. Iversen, *Science* **188**, 1084 (1975); I. Creese, D. R. Burt, S. H. Snyder, *ibid.* **192**, 481 (1976); P. Seeman, T. Lee, M. Chau-Wong, K. Wong, *Nature (London)* **261**, 717 (1976).
4. T. J. Crow *et al.*, *Adv. Biochem. Psychopharmacol.* **24**, 495 (1980); H. Y. Meltzer, D. J. Goode, V. S. Fang, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, K. F. Killam, Eds. (Raven, New York, 1978), p. 509.
5. C. C. Mao, D. L. Cheney, E. Marco, A. Revuelta, E. Costa, *Brain Res.* **132**, 375 (1977); H. Ladinsky, S. Consolo, R. Samanin, S. Algeri, F. Ponzio, *Adv. Biochem. Psychopharmacol.* **24**, 259 (1980); K. Murugaiah *et al.*, *Nature (London)* **296**, 570 (1982); B. Scatton, *Eur. J. Pharmacol.* **46**, 363 (1977).
6. F. J. White and R. Y. Wang, *Life Sci.* **32**, 983 (1983).
7. B. S. Bunney and A. A. Grace, *ibid.* **23**, 1715 (1978).
8. R. J. Baldessarini and D. Tarsy, *Annu. Rev. Neurosci.* **3**, 23 (1980).
9. A preliminary report of these findings has been presented [F. J. White and R. Y. Wang, *Soc. Neurosci. Abstr.* **8**, 921 (1982)]. In addition, L. A. Chiodo and B. S Bunney (*ibid.*, p. 482) reported preliminary evidence suggesting that clozapine selectively inactivates A10 DA neurons.
10. J. Gerlach and H. Simmelsgard, *Psychopharmacology* **59**, 105 (1978); F. N. Waldrop, R. H. Robertson, A. Vourlekis, *Comp. Psychiatry* **2**, 96 (1961); G. M. Simpson and V. Varga, *Curr. Ther. Res. Clin. Exp.* **17**, 276 (1975); A. Kirkegaard, E. Hammershoj, P. Ostergard, *Arzneim. Forsch.* **32**, 465 (1982); H. L. Klawans, C. G. Goetz, S. Perlik, *Am. J. Psychiatry* **137**, 900 (1980).
11. D. H. Mielke, D. M. Gallant, C. Kessler, *Am. J. Psychiatry* **134**, 1371 (1977); V. A. Rama Rao, J. Bailey, M. Bishop, A. Coppen, *Psychopharmacology* **73**, 77 (1981); A. A. Sugarman and J. Herrmann, *Clin. Pharmacol. Ther.* **8**, 261, (1967); F. J. Ayd, Jr., *Dis. Nerv. Syst.* **35**, 447 (1974).
12. Although it was originally believed that metoclopramide lacked antipsychotic activity [P. Borenstein and G. Bles, *Therapie* **20**, 975 (1965); B. R. S. Nakra, A. J. Bond, H. M. Lader, *J. Clin. Pharmacol.* **15**, 449 (1975)], more recent studies have found that high doses of metoclopramide can exert antipsychotic actions [M. Stanley, A. Lautin, J. Rotrosen, S. Gershon, D. Kleinberg, *Psychopharmacology* **71**, 219 (1980)]. However, since metoclopramide induces TD at much lower doses [G. D. Wood, *Br. J. Oral Surg.* **15**, 278 (1977); S. Lavy, E. Melamed, S. Penchas, *Br. Med. J.* **1**, 77 (1978)], we chose a dose regimen below the antipsychotic range but within the TD range.
13. We previously found that the HAL-induced decrease of spontaneously active A9 and A10 DA neurons was maximum after 28 days of treatment. Therefore, all drugs were compared to HAL following 28 days of treatment.
14. R. J. Baldessarini, *Chemotherapy in Psychiatry* (Harvard Univ. Press, Cambridge, Mass., 1977), pp. 12–56.
15. Four of the vehicle-treated rats were treated with the HAL vehicle (0.01*M* citric acid, *p*H adjusted to 7.2). Two rats received the CLZ and sulpiride vehicle (minimum of 0.01*M* HCl, diluted with 0.9 percent saline, *p*H adjusted to 6.0 to 7.2). Two rats received daily injections of 0.9 percent saline, which was used to dissolve all other drugs (*p*H, 6.8 to 7.2). The results for these rats were combined with those for untreated rats ($N = 4$) because there were no effects of vehicle administration.
16. Single-barrel glass micropipettes (1-μm tip; impedance, 3 to 6 megohms at 60 Hz) filled with a 2*M* NaCl solution saturated with Fast green were passed ten times in the dorsal-ventral direction from 6.0 to 8.5 mm below the cortical surface through both the ventral tegmental area (3.0 to 3.5 mm anterior to lambda and 0.5 to 0.9 lateral to the midline) and the substantia nigra zone (3.0 to 3.5 mm anterior to lambda and 2.0 to 2.4 mm lateral to the midline). Each electrode track was separated by 0.1 mm, and the sequence of tracks was constant between rats. The initial tracks in A10 and A9 were located 3.0 mm anterior to lambda and 0.5 mm lateral to the midline and 3.0 mm anterior to lambda and 2.0 mm lateral to the midline, respectively. This method allowed for accurate reproduction of the sampling procedure from rat to rat. The criteria for identifying DA neurons included a slow regular or slow bursting pattern, firing rates between 0.5 and 8.0 spikes per second, biphasic (+/−) or triphasic (+/−/+) wave forms, and spike duration above 2.5 msec [B. S. Bunney, J. R. Walters, R. H. Roth, G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* **185**, 560 (1973); A. A. Grace and B. S. Bunney, *Science* **210**, 654 (1980); R. Y. Wang, *Brain Res. Rev.* **3**, 123 (1981); F. J. White and R. Y. Wang (*6*); B. S. Bunney and A. A. Grace (*7*)]. For half of the rats in each treatment group, the order of recording was A9 and then A10; the order was reversed for the other rats. The locations of the final recorded cells were marked by passing a 25-μA current through the barrel to deposit a spot of Fast green. Locations were verified by routine histological inspection. All statistical comparisons were performed with a one-way analysis of variance followed by Dunnett's procedure for comparing multiple treatments to a single con-

trol [C. W. Dunnett, *Biometrics* **20**, 482 (1964)].
17. The irregular firing patterns were determined by obtaining interspike interval histograms of the DA neurons. These interspike interval histograms revealed increases in the number of short bursting periods and in the number of unusually long interspike intervals. Such firing patterns are rarely seen in control rats. Despite the irregular patterns, these cells met all other criteria for inclusion as DA neurons. Moreover, the A9 and A10 cells exhibiting these irregular firing patterns could usually be antidromically activated by stimulation of the caudate nucleus and nucleus accumbens, respectively. Moreover, their estimated conduction velocities were consistent with those of DA, not those of non-DA, neurons in the substantia nigra zone and ventral tegmental area. For further discussion of these findings and representative examples of interspike interval histograms, see the report by White and Wang (*6*).
18. The proposed process of depolarization inactivation suggests that APD's cause continuous excitation (or disinhibition) of DA cells, driving them above their maximal firing rate, causing excessive depolarization, and, eventually, inactivating the spike-generating mechanism of the cell, that is, raising the resting membrane potential above threshold (*6, 7*).
19. A. A. Grace and B. S. Bunney, *Soc. Neurosci. Abstr.* **8**, 481 (1982).
20. F. J. White and R. Y. Wang, *Neuropharmacology*, **22**, 669 (1983); K. Gysling and R. Y. Wang, *Brain Res.*, in press.
21. Repeated treatment with morphine at 1.0 mg/kg significantly ($P < 0.01$) increased the number of A9 and A10 DA cells per track to 120 percent (1.03 ± 0.04) and 126 percent (1.90 ± 0.21) of control, respectively. Repeated morphine treatment at 15.0 mg/kg significantly increased the number of A9 and A10 DA cells per track to 125 and 126 percent of control, respectively. Therefore, this effect of morphine was apparently not dose-related. Similar results were found in rats receiving single injections of morphine. Repeated desipramine treatment also significantly increased the number of A9 (1.20 ± 0.04) and A10 (1.97 ± 0.03) DA cells per track.
22. B. Costall and R. J. Naylor, *Psychopharmacologia* **43**, 69 (1975); R. L. Borison, J. Z. Fields, B. I. Diamond, *Neuropharmacology* **20**, 1321 (1981); S. Wilk, E. Watson, M. E. Stanley, *J. Pharmacol. Exp. Ther.* **195**, 265 (1975); G. Bartholini, *J. Pharm. Pharmacol.* **46**, 736 (1976); A. Carlsson, *Am. J. Psychiatry* **135**, 164 (1978).
23. Supported by PHS grant MH-34424 and a grant from the Scottish Rite Schizophrenia Research Program. R.Y.W. is a recipient of Research Scientist Development Award Type II MH 00378. We thank P. Dore for expert technical assistance, J. Heizer and M. Klevorn for manuscript preparation, and the following companies for generous gifts of drugs: McNeill Laboratories (HAL), Delagrange International (sulpiride), Endo (molindone), Sandoz (CLZ and TRZ), A. H. Robins (metoclopramide), Mallinckrodt (morphine), USV (desipramine), Rhone-Poulac (promethazine), and Miles Laboratories (quipazine).

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A Serological Test for Leprosy with a Glycolipid Specific for *Mycobacterium leprae*

Abstract. *A phenolic glycolipid from* Mycobacterium leprae *was purified and used as antigen in an enzyme-linked immunosorbent assay. Antibodies directed against the lipid were seen in serums from leprosy patients but not in serums from uninfected controls or patients infected with other mycobacteria, including* Mycobacterium tuberculosis. *The antibody response distinguished between the* Mycobacterium leprae *lipid and the structurally related phenolic glycolipid from* Mycobacterium kansasii. *This assay has considerable potential as a specific serodiagnostic test for leprosy infection.*

Resistance to leprosy is primarily dependent on the cell-mediated immune system, but infection with *Mycobacterium leprae* also stimulates an apparently nonprotective humoral immune response (*1–4*). Antibody levels in leprosy patients have been reported to reflect different clinical forms of the disease (*2, 4*) and to change with alterations in antigen load during chemotherapy (*4, 5*). *Mycobacterium leprae* has not yet been cultivated in vitro, and although preliminary evidence of *M. leprae*–specific antigens has been reported (*6–8*), it has not been possible to obtain these components in a form suitable for use in routine serological tests. Previous studies of humoral immunity in leprosy have therefore made use of mixtures of specific and nonspecific antigens (*4*), homogeneous cross-reactive antigens from other mycobacteria (*9, 10*), or leprosy serums absorbed with other mycobacteria to remove cross-reactive antibodies (*1*).

A phenolic glycolipid with a structure related to that of mycoside A of *Mycobacterium kansasii* (*11*) was found in *M. leprae* preparations by Draper (*12*), and its structure was elucidated by Hunter and Brennan (*13*). The unique trisaccharide component of the glycolipid (2,3-di-*O*-methyl rhamnose, 3-*O*-methyl rhamnose, and 3,6-di-*O*-methyl glucose) suggested to these authors that it may constitute a species-specific antigen, but they were unable to demonstrate serological activity using the purified lipid (*13*). Payne *et al.* (*14*), however, showed that the lipid is active in immunodiffusion experiments when it is incorporated into liposomes, and other workers indicated the possible use of the native phenolic glycolipid in an enzyme-linked immunosorbent assay (ELISA) system (*15, 16*).

We now report the development of an ELISA in which the deacylated form of the phenolic glycolipid is used. The fact that the lipid is found in large amounts in skin lesions of leprosy patients (*17*) and in tissues of experimentally infected nine-banded armadillos (*13*) suggests that it may play an important role during leprosy infection and also that it will be available in sufficient quantities for use in large-scale serological screening.

The *M. leprae* phenolic glycolipid was purified from supernatant material derived from homogenization of infected armadillo liver (*13*). Extraction by the Bligh-Dyer monophasic method (*18*) was followed by elution from a silica gel–Celite column with methanol (2 percent, by volume) in chloroform (*13*) and preparative thin-layer chromatography (TLC) on silica gel G plates developed with a mixture of chloroform and methanol (15:1, by volume). The phenolic glycolipid from *M. kansasii* was purified in the same way from bacteria grown on Middlebrook's 7H11 medium. The identities of the glycolipids were established by combined gas-liquid chromatography and mass spectroscopy of the alditol acetate derivatives generated by trifluoroacetic acid hydrolysis, followed by borohydride reduction and acetylation with acetic anhydride (*19*). Comparison of mass spectrograms of derivatives from the two glycolipids with those of standard partially methylated alditol acetates confirmed the carbohydrate composition of the *M. leprae* lipid as 2,3-di-*O*-methyl rhamnose, 3-*O*-methyl rhamnose, and 3,6-di-*O*-methyl glucose and that of the *M. kansasii* lipid as 2,4-di-*O*-methyl rhamnose, 2-*O*-methyl rhamnose, and 2-*O*-methyl fucose.

The phenolic glycolipid is a strongly hydrophobic compound and therefore difficult to use in routine immunological tests, which are generally carried out under aqueous conditions. To facilitate its use in such tests, we removed the major part of the lipid portion of the molecule by hydrolysis of the two long-chain fatty acids from the phthiocerol core in methanolic NaOH (10 percent, weight to volume) at 100°C for 16 hours. The deacylated phenolic glycolipid was recovered by preparative TLC. The more polar deacylated forms of the phenolic glycolipids from *M. leprae* and *M. kansasii* migrated more slowly than the native molecule in the organic solvent used for TLC and as a single spot, indicating complete deacylation.

The concentrations of deacylated and native phenolic glycolipid solutions were determined by assaying the carbohydrate moiety, with rhamnose used as standard (*20*). The deacylated phenolic glycolipid was prepared in aqueous suspensions by vortex-mixing the lipid (50 μg) with distilled water (1 ml) and incubating at 55°C for 15 minutes. Lipid preparations were stable for at least 1 week when stored at 0° to 4°C. For the ELISA, deacylated phenolic glycolipid was diluted with water (1:10) and added to 96-well polystyrene microtiter plates (Linbro) at 0.1 ml per well. Half of the wells on each plate were coated with the