Pharmacology Biochemistry & Behavior, Vol. 19, pp. 759–763, 1983.

Cataleptogenic Potency of the Antipsychotic Drugs is Inversely Correlated with Neuronal Activity in the Amygdaloid Complex of the Rat

GEORGE V. REBEC, JOEL GELMAN, KEVIN D. ALLOWAY

Department of Psychology, Indiana University, Bloomington, IN 47405

AND

THEODORE R. BASHORE

Department of Psychiatry, The Medical College of Pennsylvania, Philadelphia, PA 19219

Received 6 April 1983

REBEC, G. V., J. GELMAN, K. D. ALLOWAY AND T. R. BASHORE. *Cataleptogenic potency of the antipsychotic drugs is inversely correlated with neuronal activity in the amygdaloid complex of the rat.* PHARMACOL BIOCHEM BEHAV **19**(5) 759–763, 1983.—At doses known to elicit catalepsy in rats, haloperidol (1.0 and 2.0 mg/kg) and pimozide (4.0 mg/kg), injected intraperitoneally, failed to alter the spontaneous activity of neurons in the amygdaloid complex of locally anesthetized, immobilized rats. In contrast, clozapine and thioridazine, which are devoid of cataleptic effects even at high doses (10.0 and 20.0 mg/kg), caused a dramatic and prolonged increase in firing rate, whereas chlorpromazine, which produces relatively mild catalepsy at doses of 5.0 and 10.0 mg/kg, produced an intermediate response. These results, which were obtained throughout the amygdaloid complex, indicate that the cataleptogenic potency of the antipsychotic drugs is inversely correlated with their ability to accelerate neuronal activity. This finding is discussed in relation to the known mechanisms of action of these drugs on various neurotransmitter systems in the amygdaloid complex.

Amygdaloid complex Antipsychotic drugs Catalepsy Unit activity

THE therapeutic control of schizophrenia by antipsychotic medication is often complicated by the concomitant production of untoward side effects. Among the most common of these are a series of movement disorders, including rigidity and akinesia, that involve the extrapyramidal motor system [2, 5, 27]. The ability of antipsychotic drugs to produce extrapyramidal side effects (EPS), however, varies considerably. Haloperidol and pimozide, for example, routinely elicit EPS even at relatively low doses, whereas thioridazine and clozapine are virtually devoid of such effects [4, 11, 16, 33]. Chlorpromazine, a popular antipsychotic for several decades, appears to be of intermediate potency [10, 16, 26, 32]. The potency difference of these drugs in producing EPS is paralleled by their ability to cause catalepsy, an analog of EPS, in rats [12, 24, 38]. Thus, haloperidol and pimozide are very potent cataleptogenic agents, chlorpromazine is less so, and thioridazine and clozapine fail to produce catalepsy even at high doses.

Since virtually all the antipsychotics block dopamine (DA) receptors [8,25], it is unlikely that this effect alone can explain the differential abilities of these drugs to produce EPS. Instead, a regional difference in the affinity of certain antipsychotics for DA receptors has been proposed [1,3]. According to this view, cataleptogenic antipsychotics antagonize DA transmission both in the neostriatum, a part of the extrapyramidal motor system, and the nucleus accumbens, a part of the limbic system that has been implicated in schizophrenia [13,31], whereas atypical antipsychotics have a higher affinity for DA receptors in the nucleus accumbens. This regional distinction, however, is not supported by evidence that cataleptogenic and non-cataleptogenic antipsychotics produce comparable effects on DA turnover in both sites [6, 29, 30, 35, 36, 37] and that these drugs bind equieffectively to DA receptors in the neostriatum and nucleus accumbens [15]. Furthermore, these drugs produce comparable effects on neuronal activity in each site [21]. In fact, haloperidol and clozapine block the depression of firing rate produced by amphetamine and other DA agonists [20]. Thus, there appears to be little biochemical and electrophysiological support for a selective action of the non-cataleptogenic antipsychotics in the nucleus accumbens.

There is evidence, however, for a differential action of cataleptogenic and non-cataleptogenic antipsychotic drugs in the amygdaloid complex. Haloperidol, for example, exerts virtually no effect on spontaneous neuronal activity in this site, whereas clozapine produces a prolonged increase in firing rate [19]. It is conceivable, therefore, that the cataleptogenic potency of the antipsychotic drugs is inversely re-

TABLE 1

NUMBERS OF SUBJECTS AND NEURONS FROM WHICH DATA WERE OBTAINED

	Drug (mg/kg)							
	Haloperidol		Pimozide	Chlorpromazine		Clozapine	Thioridazine	
Numbers	1.0	2.0	4.0	5.0	10.0	20.0	10.0	20.0
Subjects*	3	4	6	4	4	6	3	4
Neurons	3	5	7	5	7	6	5	4

*Each rat received only one injection to avoid any effects of multiple drug or dose administrations.

lated to their ability to increase amygdaloid activity. In support of this hypothesis, we now report that haloperidol, pimozide and chlorpromazine, at doses known to elicit catalepsy in rats, produce little, if any, change in amygdaloid activity, whereas both thioridazine and clozapine cause a dramatic increase in firing rate.

METHOD

Male, Sprague-Dawley rats (300–400 g) were anesthetized with ether, mounted in a stereotaxic frame, and prepared for single unit recording as previously described [19, 20, 22]. Bilateral burr holes were drilled through the skull overlying the amydaloid complex approximately 4.6 mm anterior and 3.5 mm lateral to stereotaxic zero [14]. All points of surgical and stereotaxic contact were thoroughly infiltrated with local anesthetics (Procaine and Xylocaine) supplemented at regular intervals. The ether was withdrawn; the animals were immobilized with 2.0 mg/kg tubocurarine chloride (Lilly) and artificially respired. Body temperature (37±0.5°C), heart rate, and endtidal carbon dioxide (4.0±0.5%) were monitored throughout the recording session. Electrocorticographic recordings indicated effective local anesthesia [20,22].

Tungsten microelectrodes (3.0–8.0 MΩ impedance) were used to record single unit discharges bilaterally in the amygdaloid complex—including the basolateral, corticomedial, and central amygdaloid nuclei. Spontaneous activity, isolated to a signal-to-noise ratio of 3:1 or more, was recorded on a minute-by-minute basis for at least 20 min in order to insure a stable baseline firing rate. The mean firing rate during this period was given a value of 100%; drug-induced changes in firing rate were then recorded as a percentage of the baseline rate. This procedure permitted statistical analysis despite considerable variability among the firing rates of individual neurons recorded from different animals. Unit activity that failed to maintain a constant signal-to-noise ratio or that did not return to at least 60% of the baseline rate within 3 hr after injection was excluded. Haloperidol (McNeil), pimozide (McNeil), chlorpromazine (Sigma), clozapine (Sandoz), or thioridazine (Sandoz) was administered via a previously implanted intraperitoneal (IP) catheter.

After the completion of each experiment, the animal received a lethal overdose of sodium pentobarbital (Nembutal) and methylene blue dye was infused through the catheter to verify the accuracy of the injection site. Data were not included from animals in which dye was found outside the peritoneal cavity. Each animal received only one antipsychotic drug to avoid any residual drug effects. Electrode placements were marked by passing current through the electrode to make a small lesion. Following a transcardial perfusion with normal saline and 10% formalin, the brain was removed, frozen, sectioned and stained with cresyl violet.

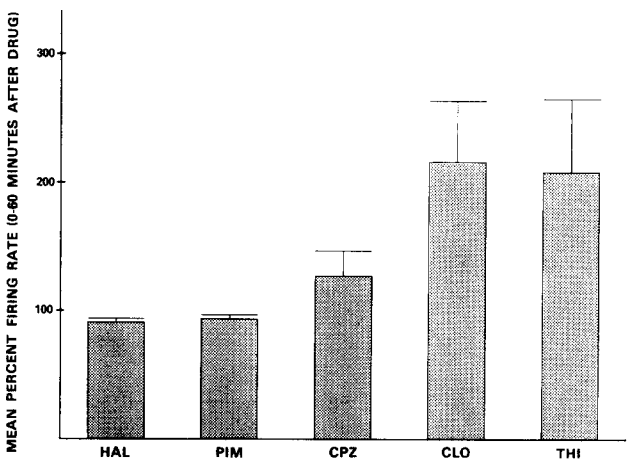

FIG. 1. Mean percent firing rate of neurons in the amygdaloid complex during the first 60 minutes after an IP injection of 2.0 mg/kg haloperiodol (HAL), 4.0 mg/kg pimozide (PIM), 10.0 mg/kg chlorpromazine (CPZ), 20.0 mg/kg clozapine (CLO), or 20.0 mg/kg thioridazine (THI). Drug-induced changes in firing rate were calculated as a percentage of the preinjection baseline rate which was defined in all cases as 100%. Brackets indicate the standard error of the mean.

RESULTS

Histological examination revealed that of the 42 neurons included for data analysis, 17 were located in the basolateral nucleus, 11 in the corticomedial nucleus, and 14 in the central nucleus. In each case, the spontaneous activity of individual neurons was slow and irregular with a rate of 1–6 spikes/sec. Biphasic action potentials having an amplitude of 300–500 μV were most common. Taken together, these data indicate no significant differences in either the waveform, amplitude, or firing pattern of individual neurons in different nuclear groups. Since neuronal activity was recorded bilaterally from each animal, the number of neurons from which data were obtained exceeded the number of rats used. Table 1 lists this information for each drug.

Figure 1 illustrates the mean percent firing rate of 29 amygdaloid neurons during the first 60 minutes after drug injection. Note the complete lack of effect of 2.0 mg/kg haloperidol and 4.0 mg/kg pimozide, and the dramatic increase in firing rate produced by 20.0 mg/kg clozapine and 20.0 mg/kg thioridazine. Chlorpromazine, at a dose of 10.0 mg/kg,

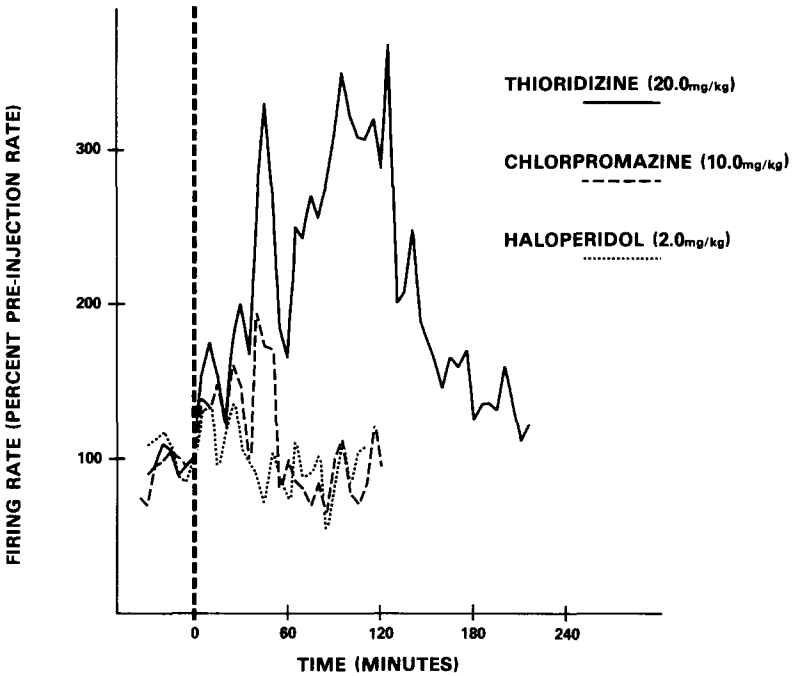

FIG. 2. Representative examples of the magnitude and time course of the neuronal response in the amygdala to the antipsychotic drugs indicated in the legend. In each case, the drug was injected at Time 0 and firing rate was plotted at 5-minute intervals as a percentage of the 100% preinjection baseline rate.

produced an intermediate response. An analysis of variance revealed that the differential effects of these neuroleptics on amygdaloid activity were highly significant, $F(4,22)=5.38$, $p<0.01$. Each nuclear group within the amygdaloid complex was represented in the data collected from each antipsychotic drug. Thus, neurons throughout the amygdaloid complex responded to these drugs in a uniform fashion.

Representative examples of the complete time-course of the neuronal response in 3 different animals are shown in Fig. 2. In a rat that received thioridazine, amygdaloid activity increased to beyond 300% of the baseline rate. In fact, neuronal activity in this case remained elevated for more than 3 hours after the injection. Chlorpromazine, on the other hand, produced a brief rise in firing rate that approached 200% of the baseline rate and that persisted for less than 1 hour. Haloperiodol produced no consistent change in neuronal activity even when firing rate was recorded for as long as 2 hours after the injection. At no time after injection did haloperidol increase amygdaloid activity to the same extent as the non-cataleptogenic antipsychotics. An analysis of group data revealed that whereas clozapine and thioridazine increased firing rate to more than 140% for periods of 40 to 230 min after injection, chlorpromazine-induced increases ranged in duration from 10 to 90 min.

It is unlikely that our data are unique to these doses since in separate groups of rats comparable results were obtained with 1.0 mg/kg haloperidol, 5.0 mg/kg chlorpromazine and 10.0 mg/kg thioridazine. Again, haloperidol produced no change in amygdaloid activity, whereas the low doses of chlorpromazine and thioridazine produced an acceleration of firing rate. In these experiments, the magnitude of the acceleration was not as pronounced as with the higher doses but a similar trend was observed. Thus, the mean firing rate during the first 60 min after an IP injection of 5.0 mg/kg chlorpromazine was 124 (±19.3)%, whereas 10.0 mg/kg thioridazine produced a mean response of 148 (±28.9)%. During the same interval after 1.0 mg/kg haloperidol, the mean firing rate was 102 (±10.2)%.

DISCUSSION

Our results confirm and extend previous suggestions that the amygdaloid complex responds differently to cataleptogenic and non-cataleptogenic antipsychotic drugs [19]. In fact, cataleptogenic potency is inversely correlated with an increase in amygdaloid activity. Thus, thioridazine and clozapine, which fail to elicit catalepsy even at relatively high doses, consistently accelerate firing rate, whereas 2.0 mg/kg haloperidol and 4.0 mg/kg pimozide routinely elicit catalepsy in rats [12, 24, 37] and, in every case, fail to alter neuronal activity. Furthermore, chlorpromazine, a relatively weak cataleptic agent at 10.0 mg/kg, produces a relatively mild acceleration of firing rate. Our results, therefore, focus attention on the amygdaloid complex as a possible mediator of drug-induced EPS. It is also possible, however, that the observed differences in amygdalar firing rates are related to other differential effects of these drugs including their anxiolytic and sedative properties. Clinically, for example, clozapine has anti-anxiety and sedative effects not found with haloperidol or chlorpromazine [11,26].

It is unlikely that the dopaminergic input to the amygdala is responsible for our data because haloperidol and pimozide, two of the most potent DA antagonists tested, were least effective in altering amygdaloid activity. Rather, a cholinergic mechanism may be involved since the cataleptogenic potency of the antipsychotic drugs is inversely re-

lated to their ability to block acetylcholine receptors. Clozapine and thioridazine, for example, are potent anticholinergic agents, whereas haloperidol and pimozide are not [28,34]. Moreover, the amygdaloid complex receives substantial cholinergic input from the nucleus basalis [9]. Thus, if cholinergic afferents normally exert an inhibitory influence on amygdaloid neurons, possibly by exciting interneurons that contain γ-aminobutyric acid (GABA), then our recordings following thioridazine or clozapine may reflect a release from tonic inhibition. An indirect decrease in the cholinergic drive to GABA-releasing neurons may also contribute to our results with non-cataleptogenic antipsychotics since cataleptogenic potency may be related to GABA turnover [7, 17, 33]. Additional experiments are now required to determine the relative contribution of cholinergic and GABAergic systems in the differential effects of the antipsychotic drugs in the amygdaloid complex.

Interestingly, the amygdala appears to be rather homogeneous in its response to each drug despite evidence for a heterogeneous distribution of neurotransmitters within this structure [23]. In fact, consistent with previous reports [18,19], spontaneous firing patterns are also uniform among different amygdaloid nuclei. It is conceivable, therefore, that rather than being driven by a single neurotransmitter, amygdaloid neurons respond to a balance among several neurotransmitter systems that ultimately determine behavior.

Finally, our results permit some speculation about the neuronal mechanisms underlying the tardive dyskinesias which develop as a consequence of long-term treatment with the cataleptogenic antipsychotics. These movement disorders have been attributed to supersensitive DA receptors in the neostriatum [2, 5, 27] but our data suggest a role for the amygdaloid complex as well. In fact, only amygdaloid neurons respond differentially to cataleptogenic and non-cataleptogenic antipsychotics suggesting that an acceleration of firing rate in this site may protect against the development of movement disorders involving the neostriatum. Consistent with this hypothesis, we have obtained preliminary evidence that the clozapine-induced increase in amygdaloid activity persists with long-term treatment (G. V. Rebec and G. D. Anderson, in preparation). Thus, it may be possible to counteract drug-induced EPS by developing new compounds that act selectively on amygdaloid neurons. Although the relative involvement of the amygdaloid complex in the therapeutic efficacy of the antipsychotic drugs remains to be elucidated, our results indicate an important and previously unrecognized role for this structure in the production of drug-induced EPS.

ACKNOWLEDGEMENTS

This research was supported by USPHS Grant DA-02451 (GVR). We thank Doris Harris Batson for expert histological analysis and Josephine Centore for invaluable editorial assistance. We also acknowledge Sandoz Pharmaceuticals (East Hanover, NJ) for providing a generous supply of clozapine and thioridazine.

REFERENCES

1. Andén, N. E. and G. Stock. Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. *J Pharm Pharmacol* **25**:345–348, 1973.
2. Baldessarini, R. J. and D. Tarsey. Dopamine and the pathophysiology of dykinesias induced by antipsychotic drugs. *Annu Rev Neurosci* **3**: 23–41, 1980.
3. Bartholini, G. Differential effect of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system. *J Pharm Pharmacol* **28**: 429–433, 1976.
4. Bowers, M. B. Thioridazine: Central dopamine turnover and clinical effects of antipsychotic drugs. *Clin Pharmacol Ther* **17**: 73–78, 1975.
5. Burki, H. R. Extrapyramidal side effects. *Pharmacol Ther* **5**: 525–534, 1979.
6. Carlsson, A. Antipsychotic drugs, neurotransmitters, and schizophrenia. *Am J Psychiatry* **135**: 164–173, 1978.
7. Costa, E., D. S. Cheney, C. C. Mao and F. Moroni. Action of antipsychotic drugs on the metabolism of γ-aminobutyric acid and acetylcholine in globus pallidus, striatum and n. accumbens. *Fed Proc* **37**: 2408–2414, 1978.
8. Creese, I. and D. R. Sibley. Receptor adaptations to centrally acting drugs. *Annu Rev Pharmacol Toxicol* **21**: 357–391, 1981.
9. Fibiger, H. C. The organization and some projections of cholinergic neurons of the mammalian forebrain. *Brain Res Rev* **4**: 327–388, 1982.
10. Glenberg, A. J. and J. C. Doller. Clozapine versus chlorpromazine for the treatment of schizophrenia: Preliminary results from a double-blind study. *J Clin Psychiatry* **40**: 238–240, 1979.
11. Gerlach, J., P. Koppelhus, E. Helweg and A. Monrad. Clozapine and haloperidol in a single-blind cross-over trial: Therapeutic and biochemical aspects in the treatment of schizophrenia. *Acta Psychiatr Scand* **50**: 410–424, 1974.
12. Honma, T. and H. Fukushima. Effects of bilateral lesions in the striatum or nucleus accumbens on the cataleptogenic activity of neuroleptics in rats. *Jpn J Pharmacol* **28**: 231–238, 1978.
13. Hornykiewicz, O. Psychopharmacological implications of dopamine and dopamine agonists: A critical evaluation of current evidence. *Annu Rev Pharmacol Toxicol* **17**: 545–559, 1977.
14. König, J. F. R. and R. A. Klippel. In: *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Baltimore: Williams and Wilkins, 1963.
15. LeFur, G., F. Guilloux and A. Uzan. In vivo blockade of dopaminergic receptors from different rat brain regions by classical and atypical neuroleptics. *Biochem Pharmacol* **29**: 267–270, 1980.
16. Lewi, P. J. Clinical and pharmacological spectral maps of neuroleptics. *Int Pharmacopsychiatry* **11**: 181–189, 1976.
17. Mao, C. C. and E. Costa. Biochemical pharmacology of GABA transmission. In: *Psychopharmacology: A Generation of Progress*, edited by M. A. Lipton, A. DiMascio and K. F. Killam. New York: Raven Press, 1978, pp. 307–318.
18. Prelevic, S., W. M. Burnham and P. Gloor. A microelectrode study of amygdaloid afferents: Temporal neocortical inputs. *Brain Res* **105**: 437–457, 1976.
19. Rebec, G. V., K. D. Alloway and T. R. Bashore. Differential actions of classical and atypical antipsychotic drugs on spontaneous neuronal activity in the amygdaloid complex. *Pharmacol Biochem Behav* **14**: 49–56, 1981.
20. Rebec, G. V., T. R. Bashore, K. S. Zimmerman and K. D. Alloway. "Classical" and "atypical" antipsychotic drugs: Differential antagonsim of amphetamine- and apomorphine-induced alterations of spontaneous neuronal activity in the neostriatum and nucleus accumbens. *Pharmacol Biochem Behav* **5**: 349–357, 1979.
21. Rebec, G. V., T. R. Bashore, K. S. Zimmerman and K. D. Alloway. Neostriatal and mesolimbic neurons: Dose-dependent effects of clozapine. *Neuropharmacology* **19**: 281–288, 1980.
22. Rebec, G. V. and S. D. Curtis. Reciprocal changes in the firing rate of neostriatal and dorsal raphe neurons following local infusions or systemic injections of D-amphetamine: Evidence for neostriatal heterogeneity. *J Neurosci*, in press.

23. Richardson, J. S. Basic concepts of psychopharmacological research as applied to the psychopharmacological analysis of the amygdala. *Acta Neurobiol Exp* **34:** 543–562, 1974.
24. Sayers, A. C., H. R. Burki, W. Ruch and H. Asper. Anticholinergic properties of antipsychotic drugs and their relation to extrapyramidal side effects. *Psychopharmacology (Berlin)* **51:** 15–22, 1976.
25. Seeman, P. Brain dopamine receptors. *Pharmacol Rev* **32:** 229–313, 1980.
26. Shopsin, B., H. Klein, M. Aaronson and M. Collora. Clozapine, chlorpromazine, and placebo in newly hospitalized, acutely schizophrenic patients. *Arch Gen Psychiatry* **36:** 657–664, 1979.
27. Simpson, G. M., E. H. Pi and J. J. Sramek. Adverse effects of antipsychotic agents. *Drugs* **21:** 138–151, 1981.
28. Snyder, S. H., D. Greenberg and H. I. Yamamura. Antischizophrenic drugs and brain cholinergic receptors. *Arch Gen Psychiatry* **31:** 58–61, 1974.
29. Stanley, M. and S. Wilk. The effect of antipsychotic drugs and their clinically inactive analogs on dopamine metabolism. *Eur J Pharmacol* **44:** 293–302, 1977.
30. Stawartz, R. J., H. Hill, S. E. Robinson, P. Settler, J. V. Dingell and F. Sulser. On the significance of the increase in homovanillic acid (HVA) caused by antipsychotic drugs in corpus striatum and limbic forebrain. *Psychopharmacologia* **43:** 125–130, 1974.
31. Stevens, J. R. An anatomy of schizophrenia? *Arch Gen Psychiatry* **29:** 177–189, 1973.
32. Swett, C., J. O. Cole, S. Shapiro and D. Slone. Extrapyramidal side effects in chlorpromazine recipients. *Arch Gen Psychiatry* **34:** 942–943, 1977.
33. Trabucchi, M., S. Govoni, G. C. Tonon and P. F. Spano. Functional interaction between receptors for dopamine antagonists and GABA central receptors. *Life Sci* **23:** 1751–1756, 1978.
34. Vanacek, J. and Z. Fink. Side effects of phenothiazines. *Act Nerv Super (Praha)* **17:** 220, 1975.
35. Waldmeier, P. C. and L. Maitre. The use of scopolamine for the estimation of the central antiacetylcholine properties of neuroleptics. *J Pharm Pharmacol* **31:** 553–555, 1979.
36. Westerink, B. H. C. and J. Korf. Influence of drugs on striatal and limbic homovanillic acid concentration in the rat brain. *Eur J Pharmacol* **33:** 31–40, 1975.
37. Wiesel, F. A. and G. Sedvall. Effect of antipsychotic drugs on homovanillic acid levels in striatum and olfactory tubercle of the rat. *Eur J Pharmacol* **30:** 364–367, 1975.
38. Worms, P. and K. G. Lloyd. Influence of GABA-agonists on neuroleptic induced catalepsy in rats. *Life Sci* **23:** 475–478, 1978.