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IN VIVO BINDING OF ^{3}H-PIMOZIDE IN MOUSE STRIATUM : EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS.

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SUMMARY

Several lines of evidence indicate that the i.v. injection of ^{3}H-pimozide results in a specific in vivo binding of the neuroleptic to dopaminergic receptors. First, ^{3}H-pimozide is preferentially accumulated in the striatum as compared to non-dopaminergic structures like the cerebellum. Second, the selective accumulation of ^{3}H-pimozide is prevented by prior administration of various neuroleptics as well as by apomorphine. Moreover, doses of antagonists which prevent this accumulation were identical to those which lead to an increased striatal HVA level. Third, ^{3}H-pimozide accumulation is not modified by the administration of a variety of non-dopaminergic agents. However, ^{3}H-pimozide binding is not prevented either by indirect dopamine agonists and is even greatly increased by d-amphetamine at high doses. The possibility that direct or indirect dopamine agonists may favour the binding of the antagonist through a modification of receptor sites is discussed.

A large body of evidence indicates that neuroleptic drugs exert their main effects by the preferential blockade of dopamine (DA) receptors in brain. For instance these drugs antagonize the behavioural effects of direct or indirect DA agonists as well as the decreased DA turnover elicited by the latter agents (1). Neuroleptic drugs have also been shown to inhibit the DA-sensitive adenylcyclase in striatal homogenates (2). More recently direct binding studies of radiolabelled DA agonists and neuroleptics have also provided strong support for this view (3-7).

Besides the information obtained from in vitro binding studies, the development of in vivo methods to study DA receptors in the living animal appears desirable. Such methods are potentially useful to avoid the artifacts resulting from the preparation of membranes and to reveal dynamic aspects of the modulation of receptor mechanisms.

For this purpose we have investigated the possible utilization of various ^{3}H-ligands of DA receptors in brain. Among these, we have studied the regional distribution of ^{3}H-apomorphine, ^{3}H-chlorpromazine, ^{3}H-haloperidol and ^{3}H-pimozide in brain following their i.v. administration (in preparation). Among the ^{3}H-neuroleptics, ^{3}H-pimozide seems to be a suitable ligand to label DA receptors in the living animal and we report here the effects of DA agonists and antagonists on its selective labelling in mouse striatum.

Independently, LADURON and LEYSEN (8) as well as HOLLT et al. (9) have demonstrated the binding of ^{3}H-pimozide and ^{3}H-spiperone in the living rodent.

MATERIALS AND METHODS

Male swiss albino mice (20-25g, Charles River, Saint-Aubin-lès-Elbeuf, France) were housed in a well ventilated room at 24°C and under artificial illumination. They were given ^{3}H-pimozide (about 0.002mg.kg^{-1}, 4.33nmol.kg^{-1}; specific activity 13Ci.mmol^{-1}, Janssen Pharmaceutica, Belgium) in the tail vein and were killed at the indicated times. Brains were removed and various areas were dissected and homogenized in 0.05M Tris-buffer pH 7.4. The radioactivity was determined in aliquots by liquid scintillation spectrometry (Intertechnique, France) in P.P.O.-P.O.P.O.P.-Toluene-Triton X 100 (16.5g-0.45g-21-11) at a counting efficiency of 32%. The tissue weight was estimated by measuring the protein content by the method of LOWRY et al. (10), assuming a 1:10 ratio of protein content to fresh weight. Results were then expressed in fmoles.g^{-1} of labelled material, taking into account the specific activity of pimozide.

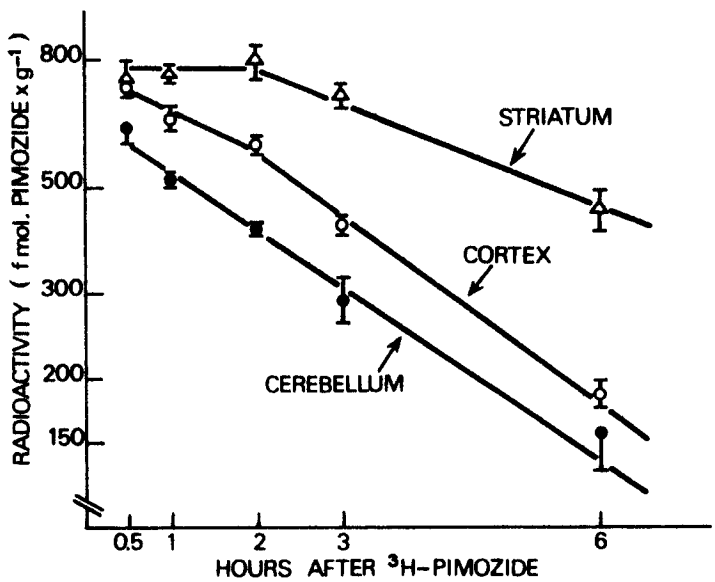
Striatal homovanillic acid (HVA) was measured according to the procedure of WESTERINK and KORF (11) to evaluate the activity of dopaminergic neurons.

RESULTS

After the intravenous administration of a low dose of ^{3}H-pimozide (0.002 mg.kg^{-1}), a small fraction is recovered in the brain 30 min later (about 0.3%) and, approximately equal concentrations are found at that time in the cerebellum, the striatum and the cortex (Fig. 1).

FIG. 1

DISTRIBUTION OF ^{3}H-RADIOACTIVITY IN DIFFERENT BRAIN AREAS AT VARIOUS TIMES AFTER THE I.V. INJECTION OF ^{3}H-PIMOZIDE.



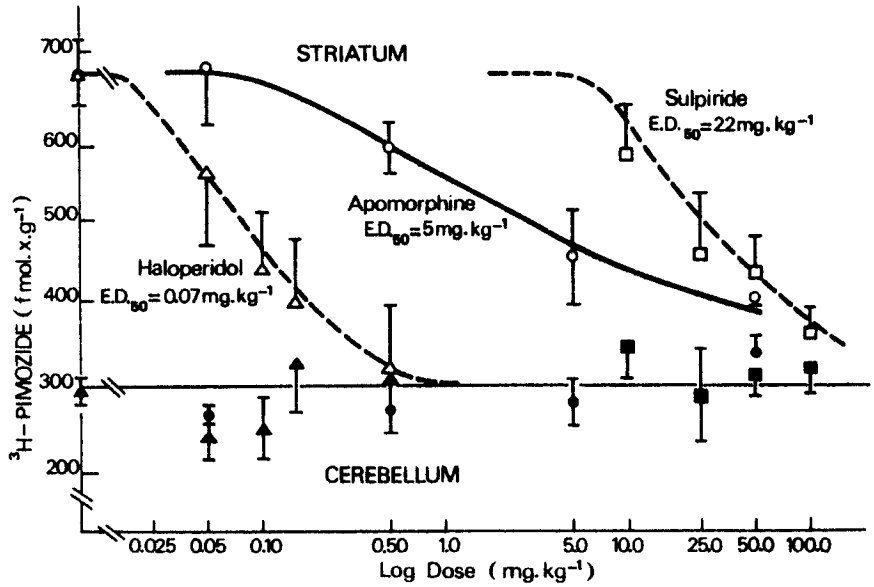
^{3}H-pimozide (1uCi, 0.002mg.kg^{-1}) was injected in the tail vein and mice sacrificed at different time intervals. Each point is the mean ± S.E.M. of 4 determinations.

However, whereas the radioactivity disappears rapidly from the cerebellum, it remains in the striatum at its initial value until 3h and, thereafter, disappears slowly. The disappearance of the radioactivity within the cortex follows a somewhat intermediate pattern (Fig. 1). At 24h the level of radioactivity is similar in all three regions (not shown). This differential retention of labelled material results, therefore, in a marked difference between the half-lives for the disappearance of ^{3}H-pimozide from the cerebellum and the striatum (2.1h and 6.0h, respectively). The difference between the radioactivity recovered in the striatum and in the cerebellum, which is barely significant 30 min after ^{3}H-pimozide administration, increases as a function of time, reaches a maximum at two hours and slowly decreases from 3h after the administration of the neuroleptic. Therefore, in all the following experiments mice were sacrificed 2h after ^{3}H-pimozide administration and the radioactivity determined in the cerebellum and in the striatum.

The efficacy of two neuroleptics, haloperidol and sulpiride, to prevent the retention of ^{3}H-pimozide in the striatum and cerebellum was compared to their efficacy to increase striatal HVA levels. Both haloperidol and sulpiride are able to prevent, in a dose-dependent manner, the retention of ^{3}H-pimozide in the striatum, without altering the level of ^{3}H-pimozide recovered in the cerebellum (Fig. 2). At high doses of the neuroleptics (0.5mg.kg^{-1} for haloperidol and 100mg.kg^{-1} for sulpiride) the radioactivity recovered in the striatum is no longer different from that recovered in the cerebellum.

FIG. 2

PREVENTION OF ^{3}H-PIMOZIDE RETENTION IN THE STRIATUM BY DOPAMINERGIC AGENTS



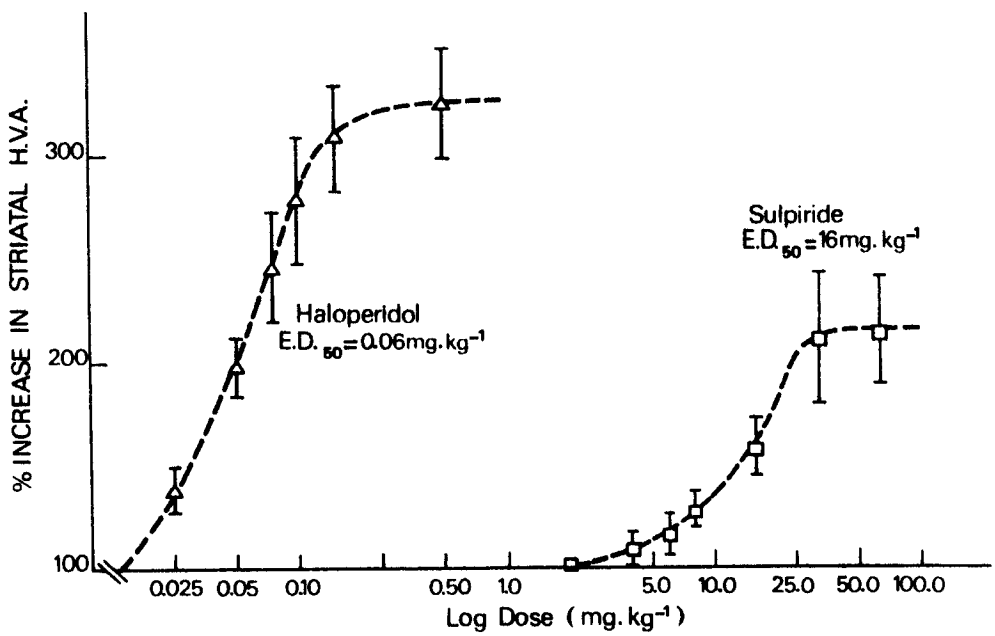
Various doses of haloperidol or sulpiride were administered i.p. 1h before the i.v. injections of ^{3}H-pimozide (1µCi, 0.002mg.kg^{-1}). Various doses of apomorphine were administered s.c. simultaneously with ^{3}H-pimozide, and mice were sacrificed 2h after the ^{3}H-pimozide injection. Results are the means + S.E.M. of 4-8 determinations. Open symbols for striatal levels and dark symbols for corresponding levels in cerebellum.

The ED_{50} for this prevention, i.e. the doses for which the difference between striatum and cerebellum is half-maximal, are 0.07mg.kg^{-1} (0.19µmol.kg^{-1}) for haloperidol and 22mg.kg^{-1} (64µmol.kg^{-1}) for sulpiride. Similarly, apomorphine, a potent and specific DA agonist, is able to prevent the retention of ^{3}H-pimozide in the striatum. While at high apomorphine dosage the radioactivity in striatum is also not significantly different from that in cerebellum, the dose-response curve is more spread out than for the neuroleptics (Fig. 2) and the ED_{50} is about 5mg.kg^{-1} (16µmol.kg^{-1}). Also both haloperidol and sulpiride are able to increase in a dose-dependent manner the striatal HVA level with ED_{50} of about 0.06mg.kg^{-1} and 16mg.kg^{-1}, respectively (Fig. 3).

Besides apomorphine, indirect DA agonists were tested : neither L-DOPA in combination with an inhibitor of decarboxylases in peripheral tissues nor d-amphetamine (1mg.kg^{-1}) modify the retention of ^{3}H-pimozide (Table I). Surprisingly, administration of amphetamine in higher doses results in a marked increase in the striatal level of ^{3}H-pimozide without altering the cerebellar level ; hence the difference between the two regions is enhanced by about 70%.

FIG. 3

EFFECTS OF HALOPERIDOL AND SULPIRIDE AT VARIOUS DOSES ON STRIATAL HVA LEVELS



Striatal HVA levels were determined 90 min after the i.p. administration of haloperidol or sulpiride. Results, expressed in percentage of control values (0.95 ± 0.4µg.g^{-1} in the experiment with haloperidol and 0.66 ± 0.04µg.g^{-1} in the experiment with sulpiride) are the means ± S.E.M. of 6-12 determinations.

TABLE I

Effects of dopaminergic agonists on the ^{3}H-pimozide levels in the striatum and cerebellum

AGONISTS	CEREBELLUM fmol.g^{-1}	STRIATUM fmol.g^{-1}	Δ fmol.g^{-1}
Saline	301 ± 26	787 ± 67	485 ± 43
L-DOPA + Ro 4 4602 200mg.kg^{-1}	397 ± 30	891 ± 191	485 ± 48
d-amphetamine 1mg.kg^{-1}	392 ± 20	867 ± 48	475 ± 33
d-amphetamine 5mg.kg^{-1}	314 ± 22	1138 ± 119*	804 ± 102**
d-amphetamine 20mg.kg^{-1}	325 ± 41	1140 ± 154*	815 ± 117**

d-amphetamine was administered s.c. and L-DOPA i.p. immediately before the i.v. injection of ^{3}H-pimozide (1μCi ; 0.002mg.kg^{-1}) and mice were killed 2h latter. Δ represents the difference between ^{3}H-pimozide levels in striatum and cerebellum. Means ± S.E.M. of 4-10 experiments. $p < 0.05$; ** $p < 0.01$ as compared to controls.*

TABLE II

Effects of various drugs on the ^{3}H-pimozide levels in the striatum and cerebellum

DRUGS	DOSE mg.kg^{-1}	CEREBELLUM fmol.g^{-1}	STRIATUM fmol.g^{-1}	Δ fmol.g^{-1}
Saline	-	327 ± 20	719 ± 63	384 ± 26
Propranolol	5.0	301 ± 39	698 ± 76	397 ± 37
Clonidine	1.0	299 ± 2	637 ± 26	340 ± 26
Promethazine	10	397 ± 61	745 ± 113	351 ± 59
Methysergide	1.0	327 ± 39	756 ± 93	429 ± 61
LSD	1.0	301 ± 35	650 ± 48	379 ± 46
Scopolamine	2.5	334 ± 41	784 ± 63	453 ± 28
Chlordiazepoxide	20	316 ± 20	763 ± 59	446 ± 39
Baclofen	20	308 ± 30	620 ± 30	314 ± 39
Morphine	50	407 ± 30*	1016 ± 74**	609 ± 48**

All drugs administered i.p. simultaneously with ^{3}H-pimozide (1uCi, 0.002 mg.kg^{-1}, i.v.) and mice killed 2h later. Δ represents the difference between ^{3}H-pimozide levels in striatum and in cerebellum. $p < 0.05$; ** $p < 0.01$ as compared to controls. Means ± S.E.M. of 5-10 experiments.*

Among a great variety of drugs, adrenergic agents (propranolol or clonidine), serotoninergic agents (methylsergide or LSD), a cholinergic agent (scopolamine) or miscellaneous agents (promethazine, chlordiazepoxyde, baclofen) do not modify significantly the retention of ^{3}H-pimozide in either the striatum or the cerebellum (Table II). On the other hand, ^{3}H-pimozide retention is significantly increased both in striatum and cerebellum of morphine-treated mice and the difference between the two regions is also significantly higher than in the controls.

DISCUSSION

In the present work, we have investigated the possibility that the retention of ^{3}H-radioactivity in various brain areas following the i.v. administration of ^{3}H-pimozide at a very low dose reflects the binding of the neuroleptic drug to selective sites. No attempt was made to identify the nature of ^{3}H-radioactivity which, presumably, is mainly attributable to the unchanged drug since metabolic studies have shown that, 2h after i.v. administration, 90% of total ^{3}H in brain is present as unchanged ^{3}H-pimozide (8 ; P. LADURON, personal communication). Hence, the total ^{3}H-radioactivity in brain areas was always referred to as ^{3}H-pimozide.

Several lines of evidence indicate that retention of ^{3}H-pimozide in brain can be regarded as the sum of two components : a non-specific retention and the binding to DA receptors.

First, by analysis of the time-course of the disappearance of ^{3}H-pimozide from brain, regions rich in DA receptors (striatum) can be distinguished from regions where they are believed to be absent (cerebellum). Thirty minutes after ^{3}H-pimozide administration the drug levels in various brain areas are not markedly different. However, the rapid disappearance of ^{3}H-pimozide from the cerebellum, contrasting with a greater retention in striatum, progressively reveals the existence of a selective labelling in this region. Similar differential distribution of ^{3}H-pimozide have been recently found in rat brain and interpreted in the same way (8). In the cortex, a region where DA nerve-endings are present (12) but in smaller number than in the striatum, a somewhat intermediate pattern is observed. We have not tried to relate the retention in mouse cortex to the presence of dopaminergic endings in more discrete areas but the use of ^{3}H-spiperone has apparently enabled LADURON and LEYSEN (8) to do so in the rat.

Hence, it can be assumed that the ^{3}H-pimozide levels in the cerebellum reflect a non-selective retention and that the difference between levels in striatum and cerebellum represents the binding to DA receptors.

This view is supported by the actions of a variety of agents on the selective labelling in the striatum, expressed in Tables I and II by the difference in striatal and cerebellar levels. On the one hand, none of the non-dopaminergic agents tested modified the selective labelling in striatum or the level in cerebellum. On the other hand, neuroleptics of various chemical classes such as haloperidol, sulpiride (Fig. 2) and also chlorpromazine (in preparation) as well as apomorphine, a potent DA agonist, are able to completely prevent the selective retention of ^{3}H-pimozide in the striatum without modifying the level in cerebellum. Moreover, the ED_{50} of haloperidol and sulpiride for the prevention of selective binding were closely similar to their ED_{50} for the increase in striatal HVA level, indicating that binding of ^{3}H-pimozide occurs at the sites whose blockade activates the negative feedback mechanism responsible for the increased DA release. On the other hand, the ED_{50} of apomorphine (5mg.kg^{-1}) is rather high as compared to its ED_{50} in other tests like the elicitation of a stereotyped behaviour (13) or the decrease in HVA level (14) in mouse. This discrepancy can be explained by the short half-life of the alkaloid in mouse brain, i.e. 15 min (14), as well as by its relatively small affinity for the binding sites of neuroleptics, as measured in vitro

(4, 5, P. LADURON, personal communication).

Taken together all these data clearly demonstrate that the selective retention of ^{3}H-pimozide in mouse striatum results from its binding to sites which possess some of the characteristics of DA receptors. It was therefore of interest to check whether an increased release of DA in the striatum was able to prevent this selective binding. In fact, the administration of indirect DA agonists, L-DOPA or d-amphetamine, does not prevent this binding - an observation which can be related with the low efficacy of DA to inhibit the binding of ^{3}H-neuroleptic in vitro (4, 5).

Surprisingly, when amphetamine was administered in high doses, ^{3}H-pimozide was markedly increased in striatum. It is unlikely that such an effect is due to a change in the availability of ^{3}H-pimozide because its level in cerebellum remained the same. A possible explanation could be that the enhanced DA release with amphetamine administration results in a modification of the binding sites for the neuroleptics, due to an increase in either their affinity or number. This hypothesis could also explain the peculiar aspect of the curve of protection by apomorphine. In contrast with the two parallel protection curves for the neuroleptics (Fig. 2), that of apomorphine stretches over a large scale of doses, suggesting a prevention of ^{3}H-pimozide binding which is not purely competitive : in addition to a competitive inhibition of binding, apomorphine would elicit the same modifications of the binding sites as postulated for endogenous DA released by amphetamine. The postulated changes in sites binding the antagonists that are elicited by the agonists could be accounted for by the existence of distinct interconverting conformations of the DA receptor, a model already developed for other brain receptors (15). It is not yet clear whether our interpretation is also true for the effect of morphine (Table II), in view of the complex interrelationships between morphine and the DA systems (16, 17).

In conclusion it appears that the use of labelled ligands for in vivo experiments opens new possibilities in the field of cerebral receptor research, inasmuch as it may well reveal dynamic modifications not easily detectable by other approaches.

REFERENCES

1. G. SEDVALL, B. UVNAS and Y. ZOTTERMAN, Antipsychotic drugs : pharmacodynamics and pharmacokinetics, p. 1-459, Pergamon Press, London (1976).
2. J.W. KERABIAN, PETZOLD G.L. and P. GREENGARD, Proc. Natl. Acad. Sci. (USA) 69, 2145-2149 (1972).
3. D.R. BURT, S.J. ENNA, I. CREESE and S.H. SNYDER, Proc. Natl. Acad. Sci. USA 72, 4655-4659 (1975).
4. I. CREESE, D.R. BURT and S.H. SNYDER, Life Sci. 17, 993-1002 (1975).
5. P. SEEMAN, M. CHAU-WONG, J. TEDESCO and K. WONG, Proc. Nat. Acad. Sci. (USA) 72, 4376-4380 (1975).
6. P. SEEMAN, T. LEE, M. CHAU-WONG and K. WONG, Nature 261, 717-719 (1976).
7. P. SEEMAN, T. LEE, M. CHAU-WONG, J. TEDESCO and K. WONG, Proc. Natl. Acad. Sci. (USA) 73, 4354-4358 (1976).
8. P. LADURON and J. LEYSEN, Biochem. Pharmacol. 26, 1003-1007 (1977).
9. V. HOLLT, A. CZLONKOWSKI and A. HERZ, Brain Res. 130, 176-183 (1977).
10. O.H. LOWRY, N.J. ROSEBROUGH, A.L. FARR and R.J. RANDALL, J. Biol. Chem. 193, 265-275 (1951).
11. B.H.C. WESTERINK and J. KORF, Biochem. Med. 12, 106-115 (1975).
12. A.M. THIERRY, G. BLANC, A. SOBEL, L. STINUS and J. GLOWINSKI, Science 182, 499-501 (1973).
13. J. COSTENTIN, P. PROTAIS and J.C. SCHWARTZ, Nature 257, 405-407 (1975).
14. M.P. MARTRES, J. COSTENTIN, M. BAUDRY, H. MARCAIS, P. PROTAIS and J.C. SCHWARTZ, Brain Res., in press (1977).

15. S.H. SNYDER, Biochem. Pharm. 24, 1371-1374 (1975).
16. K. KUSCHINSKY, Arzneim. Forsch. (Drug Res.) 26, 563-567 (1976).
17. H. POLLARD, C. LLORENS-CORTES and J.C. SCHWARTZ, Nature 268, 745-747 (1977).