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PRELIMINARY COMMUNICATION

SPECIFIC IN VIVO BINDING OF NEUROLEPTIC DRUGS IN RAT BRAIN

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Neuroleptic drugs are known to increase dopamine turnover not only in both the basal ganglia [1] and mesolimbic system [2] but also in the mesocortical system [3,4]. These effects have been interpreted in terms of blockade of dopamine receptors (cfr. ref. 5). The recent findings that neuroleptic drugs can inhibit dopamine-sensitive adenylate cyclase [6] and specifically bind to receptors in dopaminergic regions of the brain [7,8], have provided more direct evidence to support the hypothesis that neuroleptic drugs block dopamine receptors. Since the enzyme and the specific binding sites have been reported to be localized in different subcellular structures from rat striatum [9], they might be considered as two possible targets capable of eliciting different biochemical and behavioral effects. The purpose of the present investigations was to examine to what extent these receptors are really involved in the pharmacological activity of neuroleptic drugs. Therefore, an in vivo assay was developed using two different neuroleptic drugs ; spiperone, a butyrophenone derivative and pimozide, the prototype of the diphenylbutylpiperidines.

Male Wistar rats (250 g) were given ^{3}H spiperone (0.005 mg.kg^{-1} spec.act. 9 Ci/mmol or ^{3}H pimozide (0.02 mg.kg^{-1} spec.act. 13 Ci/mmol Janssen Pharmaceutica) by intravenous injection. Two hours later, the animals were killed by decapitation. Brains were removed and various areas were dissected. They were combusted in a tissue sample oxidizer and the radioactivity was measured in a liquid scintillation spectrometer.

Table 1 shows the regional distribution of ^{3}H spiperone and ^{3}H pimozide in the rat brain. Both drugs were found to be more specifically taken up in the dopaminergic areas of the rat brain, thus confirming earlier findings [10] in the dog when using ^{3}H haloperidol and ^{3}H pimozide. As shown in table 1, the difference in the labelled drug content between the brain areas known to contain dopaminergic terminals and the other ones (e.g. parietal cortex, cerebellum and brain remainder) was much more pronounced with spiperone than with pimozide. For instance, there was about 10 times more ^{3}H spiperone in the nucleus accumbens than in the cerebellum. Although less marked, a higher retention capacity in the dopaminergic areas was also observed using ^{3}H pimozide. It is noteworthy that the frontal cortex was found to contain more ^{3}H spiperone than the parietal side, and this may explain the much higher HVA increase in the former than in the latter after haloperidol treatment [4]. The substantia nigra where dopamine release has recently been demonstrated [11], also appeared to possess the higher retention capacity for neuroleptic drugs. The regional distribution of ^{3}H spiperone and of ^{3}H pimozide closely parallels that of the neuroleptic receptor binding sites when measured in in vitro conditions. This suggests that the different retention capacity of a given region is directly related to the number of receptor sites involved.

^{3}H spiperone was also found to be specifically displaced by a larger dose of unlabelled com-

Table 1. Regional distribution of labelled drugs in rat brain 2 hours after i.v. injection of ^{3}H spiperone (0.005 mg.kg^{-1}) and ^{3}H pimozide (0.02 mg.kg^{-1}).

Brain region	pg.mg^{-1} ± SEM (n=6) Spiperone	pg.mg^{-1} ± SEM (n=5) Pimozide
Nucleus accumbens	2.79 ± 0.38	6.36 ± 0.19
Striatum	2.50 ± 0.15	7.63 ± 0.49
Substantia nigra	1.71 ± 0.30	4.11 ± 0.19
Tuberculum olfactorium	1.59 ± 0.08	5.06 ± 0.37
Frontal cortex	1.02 ± 0.05	4.27 ± 0.19
Parietal cortex	0.74 ± 0.06	4.05 ± 0.19
Cerebellum	0.29 ± 0.02	2.65 ± 0.25
Rest of the brain	0.77 ± 0.06	3.35 ± 0.21

pound in the dopamine containing areas. Indeed fig.1 shows that labelled spiperone decreased much more rapidly in the striatum when unlabelled spiperone was injected one hour after the radioactive drug. In contrast to this, no displacement was detectable in the cerebellum. In other dopaminergic areas such as the nucleus accumbens, the tuberculum olfactorium and

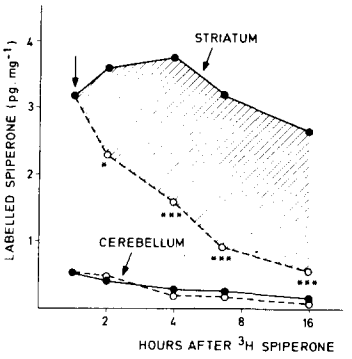

Fig. 1. In vivo displacement of ^{3}H spiperone (0.005 mg.kg^{-1} i.v.) in rat striatum and cerebellum. In the first group (●——●), rat brains were removed at different time intervals after injection of labelled spiperone. In the second group (○---○), 0.63 mg.kg^{-1} unlabelled spiperone was injected intravenously one hour after ^{3}H spiperone. The hatched part represents displaceable ^{3}H spiperone. Each point is the mean of 4 determinations. Significant differences from control values are indicated by * $p < .05$ *** $p < .001$. (P : student t-test)

even, but to a lesser extent, the frontal cortex, we have observed a similar displacement after a large dose of unlabelled drug using either ^{3}H spiperone or ^{3}H pimozide (in preparation). Thus, only those molecules which are specifically bound to the receptors of the dopaminergic structures can be displaced from their binding sites whereas those which are bound aspecifically, cannot.

A third line of evidence for specific in vivo binding of neuroleptic drugs was provided by the study of the subcellular localization of ^{3}H spiperone in rat striatum and cerebellum. Brain samples were fractionated by differential centrifugation according to the analytical five-fraction procedure [12, 13]. In order to assess the composition of the subcellular fractions thus obtained, several marker enzymes were determined according to methods previously described [12, 13].

Fig.2 shows the distribution pattern of ^{3}H spiperone in fractions obtained from rat striatum and cerebellum homogenates. Ninety percent of the radioactivity was recovered in particulate fractions in the striatum against only 49 percent in the cerebellum. ^{3}H spiperone was mainly found to have been enriched in the P (microsomal) fraction (42 %) in the striatum but not in the cerebellum (7 %). It is noteworthy that the ^{3}H spiperone profile in the striatum closely parallels that of the neuroleptic receptor as previously reported [9], when assayed in in vitro conditions. In contrast to this, in both regions, its profile markedly differed from that of cytochrome oxidase (mitochondrion) of lactate dehydrogenase (synaptosome) of N-acetyl-β-D glucosaminidase (lysosome) and even of dopamine-sensitive adenylate cyclase as already described [13]. Consequently, none of these structures seems

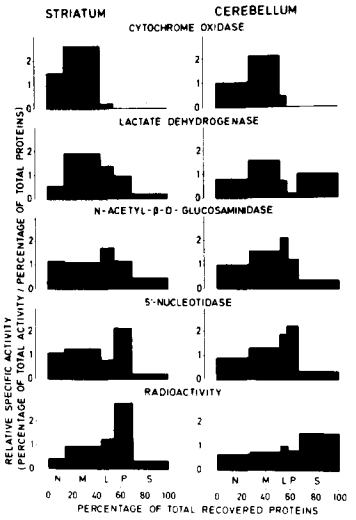

Fig. 2. Distribution pattern of radioactivity and marker enzymes in subcellular fractions of rat striatum and cerebellum obtained by differential centrifugation, two hours after i.v. injection of ^{3}H spiperone (0.005 mg. kg^{-1}). The absolute amount of ^{3}H spiperone in the starting material was 2.9 ng g^{-1} in the striatum and 0.4 ng g^{-1} in the cerebellum, while their distribution in percent was respectively 6 and 17 in N (nuclear fraction), 28 and 19 in M (heavy mitochondrial fraction), 14 and 6 in L (light mitochondrial fraction), 42 and 7 in P (microsomal fraction), 10 and 51 in S (supernatant).

to be the main target in vivo for neuroleptic drug activity. In control experiments in which labelled spiperone was added to the starting homogenate at a concentration corresponding to that normally found in the homogenate under in vivo conditions, only 18 % and 40 % of ^{3}H spiperone were respectively recovered in the P and S fractions in the striatum. On the contrary, the distribution pattern in the cerebellum was identical in both conditions indicating that in this brain region, no specific binding occurred in vivo, a fact which is quite compatible with the absence of neuroleptic receptors in this area. Therefore, one may conclude that the ^{3}H spiperone enrichment in the P fraction corresponds to a specific in vivo binding on neuroleptic receptors. Moreover, although we cannot quite rule out that a small amount of spiperone should also be associated with structures bearing dopamine-sensitive adenylate cyclase, the inhibition of this enzyme does not seem to be of great importance for the antipsychotic activity of neuroleptic drugs. We believe that the inhibition of the dopamine-sensitive adenylate cyclase is only involved in the mechanism of action of neuroleptic drugs endowed with sedative effects.

Throughout the present work, radioactivity was always considered either as unchanged spiperone or as unchanged pimozide. Metabolic studies have shown that 2 hours after intravenous injection 90 percent of ^{3}H spiperone and ^{3}H pimozide were found in the rat brain as unchanged drug [14]. Moreover the amount of pimozide metabolites per mg.tissue was similar in all the brain regions for 24 hours after injection. These metabolites were never found to have been particulate-bound but well contained in the supernatant fraction (J. Heykants ; unpublished results).

Spiperone seems to be particularly appropriate for such in vivo studies ; indeed, recent in vitro binding experiments have shown that, when compared to haloperidol, spiperone has a slower dissociation rate, a higher affinity for neuroleptic receptor but a lower one for aspecific sites (unpublished results). This is also true for pimozide except that its aspecific binding was relatively higher, a fact which can explain why the retention of ^{3}H pimozide in the cerebellum was relatively more pronounced than that of ^{3}H spiperone (table 1).

In the present study, three lines of evidence were provided to demonstrate a specific in vivo binding for neuroleptic drugs. First, these drugs were specifically taken up in the dopaminergic areas of the brain so that the higher retention capacity in these regions, when compared to that in non dopaminergic areas, (e.g. the cerebellum), might be taken as an index for specific in vivo binding. However, specific distributions are only possible if a very low dose of the drug is used in a way that the high affinity specific binding is predominant, thus not masked by the low affinity aspecific binding [15]. Secondly, larger doses of unlabelled drug could displace the labelled drug in vivo only in the dopaminergic areas. Recent results have shown that apomorphine and aminotetralin derivatives can also be used in these displacement experiments, a fact which provides further evidence concerning the dopaminergic nature of the neuroleptic receptor. Thirdly, specificity for this in vivo binding was further assessed at the subcellular level since the labelled neuroleptic drug was found to be specifically enriched in the microsomal fraction of the dopamine containing areas.

The present results support the view that, in vivo, the neuroleptic receptor may be a more important target for neuroleptic drugs than dopamine-sensitive adenylate cyclase.

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