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Brain σ receptors labelled by [^{3}H]nemonapride

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Abstract

Binding of [^{3}H]nemonapride and [^{3}H]raclopride was examined in the brain areas of three species (rat, cow and human). The results indicated that [^{3}H]nemonapride binding is inhibited by selective σ receptor ligands in frontal cortex, striatum and cerebellum. Only the striatum showed significant dopaminergic sites as defined by sulpiride. Use of the subtraction method of [^{3}H]nemonapride minus [^{3}H]raclopride binding as a measure of D_4 dopamine receptor binding may, therefore, also include a σ receptor component.

Keywords: Dopamine D_4 receptor; Raclopride

The successful cloning of dopamine D_4 receptors and the demonstration that clozapine binds with high affinity to these dopamine receptors started a new round of interest in the role of dopamine receptors in schizophrenia (Van Tol et al., 1992). In the study of post-mortem human schizophrenic brains, Seeman and co-workers measured dopamine D_4 receptor densities by a subtraction method (Seeman et al., 1993) since no ligand was available which bound specifically to dopamine D_4 receptors alone. This subtraction method was adapted by several subsequent investigations with controversial results (Murray et al., 1995; Reynolds and Mason, 1995; Seeman et al., 1995; Sumiyoshi et al., 1995). The method uses the differential between the saturating concentration binding of [^{3}H]-raclopride (or [^{125}I]epidepride) and [^{3}H]nemonapride in post-mortem schizophrenic brains. The subtraction method assumes that [^{3}H]raclopride labels dopamine D_2 and D_3 sites while [^{3}H]nemonapride labels dopamine D_2, D_3 and D_4 sites. We report in this communication that [^{3}H]-nemonapride labels brain σ receptors.

[^{3}H]Raclopride (74.0–85.6 Ci/mmol) and [^{3}H]-nemonapride (86.0 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA). Butaclamol, PPAP (*R*(−)-*N*-(3-phenyl-*n*-propyl)-1-phenyl-2-aminopropane hydrochloride) and *S*(−)sulpiride were purchased from Research Biochemical International (Natick, MA). Common chemicals were from Sigma Chemical Co. (St. Louis, MO) and Calbiochem (La Jolla, CA). Human post-mortem brain tissues were obtained from the National Neurological Research Specimen Bank (West Los Angeles Veterans Administration Medical Center, Westwood, Los Angeles, CA; c/o Dr. W.W. Tourtellotte). Calf brain tissues were purchased from local slaughterhouses (Los Angeles, CA). Rat brain tissues were purchased from Pel Freez Biologicals (Rogers, AK). Frozen tissue was homogenized (glass-Teflon; 10 up-down strokes) in D_4 buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 120 mM NaCl) and frozen at −80°C until use. On the day of the experiment, tissue was washed twice by centrifugation at 30 996 × *g* and resuspended in D_4 buffer to give 1.2 mg original wet weight per tube for cow and human tissue, and 0.8 mg per tube for rat tissue. Binding of tissue homogenates to either 1 nM [^{3}H]nemonapride or 6 nM [^{3}H]raclopride (final concentration) was done in polypropylene tubes for 3 h at room temperature (22°C) in the case of [^{3}H]nemonapride and for 2 h (22°C) in the case of [^{3}H]raclopride, before separation of bound from free ligand by vacuum filtration through GF/B glass fiber filters (Whatman, UK). Assay volume per tube was 0.3 ml. Gpp(NH)p (sodium salt) was not added to the washed tissue since our own results (unpublished) indicated that it enhanced [^{3}H]raclopride binding only if the tissue was not washed.

As shown in Table 1, [^{3}H]nemonapride and [^{3}H]-raclopride gave different amounts of specific binding in

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Table 1
Regional binding profile

Species	Region	Ligand	Specific binding (fmol/mg wet weight)
(A) Specific binding defined with 10 μM S(−)sulpiride			
Rat	Striatum	[^{3}H]nemonapride	13.4 ± 0.4
		[^{3}H]raclopride	4.0 ± 0.5
	Frontal cortex	[^{3}H]nemonapride	0.7 ± 0.2
		[^{3}H]raclopride	0.6 ± 0.3
	Cerebellum	[^{3}H]nemonapride	0.6 ± 0.2
		[^{3}H]raclopride	0.7 ± 0.3
Cow	Striatum	[^{3}H]nemonapride	6.3 ± 0.9
		[^{3}H]raclopride	3.4 ± 0.8
	Frontal cortex	[^{3}H]nemonapride	2.1 ± 0.6
		[^{3}H]raclopride	0.2 ± 0.1
	Cerebellum	[^{3}H]nemonapride	2.3 ± 0.8
		[^{3}H]raclopride	0.5 ± 0.2
Human	Striatum	[^{3}H]nemonapride	6.9 ± 1.1
		[^{3}H]raclopride	4.5 ± 1.3
(B) Specific binding defined with 1 μM PPAP			
Cow	Striatum	[^{3}H]nemonapride	12.6 ± 1.4
		[^{3}H]raclopride	0.6 ± 0.1
	Frontal cortex	[^{3}H]nemonapride	7.3 ± 0.6
		[^{3}H]raclopride	0.2 ± 0.1
	Cerebellum	[^{3}H]nemonapride	10.2 ± 0.4
		[^{3}H]raclopride	0.2 ± 0.2
Human	Striatum	[^{3}H]nemonapride	6.2 ± 1.2
		[^{3}H]raclopride	0.5 ± 0.3
	Frontal cortex	[^{3}H]nemonapride	8.9 ± 1.2
		[^{3}H]raclopride	0.04 ± 0.04
	Cerebellum	[^{3}H]nemonapride	9.7 ± 0.4
		[^{3}H]raclopride	0.5 ± 0.3
(C) Specific binding defined with 10 μM (+)-butaclamol			
Rat	Striatum	[^{3}H]nemonapride	20.8 ± 1.4
		[^{3}H]raclopride	6.1 ± 0.1
	Frontal cortex	[^{3}H]nemonapride	5.1 ± 0.7
		[^{3}H]raclopride	1.6 ± 0.6
	Cerebellum	[^{3}H]nemonapride	3.2 ± 0.8
		[^{3}H]raclopride	2.7 ± 0.7
Cow	Striatum	[^{3}H]nemonapride	17.3 ± 0.5
		[^{3}H]raclopride	3.2 ± 0.3
	Frontal cortex	[^{3}H]nemonapride	7.2 ± 0.2
		[^{3}H]raclopride	1.5 ± 0.1
	Cerebellum	[^{3}H]nemonapride	7.1 ± 0.8
		[^{3}H]raclopride	1.8 ± 0.1
Human	Striatum	[^{3}H]nemonapride	11.1 ± 0.7
	Frontal cortex	[^{3}H]nemonapride	8.2 ± 0.1
	Cerebellum	[^{3}H]nemonapride	7.6 ± 0.4

Data expressed as mean ± S.E.M. (n = 3–6 per group).

striatum, frontal cortex and cerebellum, depending on the type of compound used to define specific binding. The σ receptor selective ligand PPAP demonstrated minimal competition for [^{3}H]raclopride binding in any of the regions studied (IC_{50} > 1 μmol). On the other hand, PPAP potently competed for [^{3}H]nemonapride binding [IC_{50} values: 53.3 ± 14.9 nM, and 33.3 ± 2.1 nM in human and calf striatum, respectively (mean ± S.E.M., n = 3/group); 38.0 ± 6.7 nM and 38.2 ± 6.5 nM in human and calf frontal cortex, respectively (n = 3/group); 49.1 ± 17.2 nM and 43.5 ± 7.4 nM in human and calf cerebellum, respectively (n = 3/group)]. At 10 μM concentration, PPAP competed for approximately 50% of human and calf striatal [^{3}H]nemonapride total binding, 80% of human and calf frontal cortical total binding, 70% of human cerebellum and 80% of calf cerebellum total [^{3}H]nemonapride binding.

While (+)-butaclamol was not potent in competing for [^{3}H]nemonapride binding in the cerebellum and frontal cortex of both human and calf (i.e., IC_{50} values: 3140 ± 500 nM and 2730 ± 220 nM in human cerebellum and frontal cortex, respectively, mean ± S.E.M., n = 3/group), (−)-butaclamol was very effective (IC_{50} values: 210 ± 5 nM and 290 ± 23 nM in human cerebellum and frontal cortex, respectively, n = 3/group). In human striatum, the high affinity components of (+) and (−)-butaclamol competition curves were more similar in competing for [^{3}H]nemonapride binding (IC_{50} values: 330 ± 38 nM for (−)-butaclamol and 790 ± 170 nM for (+)-butaclamol, n = 3/group). This is reasonable considering that both dopaminergic [which has higher affinity for (+)-butaclamol] and σ sites [which have higher affinity for (−)-butaclamol] are present in large quantities in this area.

In summary, the data indicate that a differential could be obtained between [^{3}H]nemonapride and [^{3}H]raclopride binding in rat, calf and human striatal tissues, as well as in the frontal cortex and cerebellum depending on the compound used to define specific binding. Seeman et al. (1993), Sumiyoshi et al. (1995) and Reynolds and Mason (1995) used S(−)sulpiride to define specific binding while Seeman et al. (1995) also used 1 μM haloperidol for this purpose; Murray et al. (1995) used 10 μM (+)-butaclamol to define specific binding of [^{3}H]raclopride and [^{3}H]nemonapride. While we were able to confirm that use of sulpiride or (+)-butaclamol gives a differential for [^{3}H]raclopride and [^{3}H]nemonapride binding, differentials were also obtainable by using the non-dopaminergic compounds PPAP and (−)-butaclamol. Both PPAP and (−)-butaclamol bind to brain σ receptors (Glennon et al., 1990; Weissman et al., 1988). Both showed affinities consistent with σ binding and under our D_4 binding conditions, both showed competition for a substantial proportion of total [^{3}H]nemonapride binding (i.e., 50–80% in all areas studied; data not shown). It should also be noted that (+)-butaclamol (at 10 μM concentration) and haloperidol should compete for both σ and dopaminergic sites according to the literature (Weissman et al., 1988). Thus, [^{3}H]nemonapride is not specific in labelling brain dopamine receptors. In fact, it labels sites displaceable by σ ligands.

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