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80% of muscarinic receptors expressed by the NB-OK 1 human neuroblastoma cell line show high affinity for pirenzepine and are comparable to rat hippocampus M1 receptors

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The NB-OK 1 human neuroblastoma cell line expressed muscarinic cholinergic receptors that could be labeled with *N*-[^{3}H]methylscopolamine (a nonselective antagonist). 80% of these receptors showed high affinity for pirenzepine, i.e. belonged to the M1 subtype found in neuronal tissues. Their binding properties were identical to those of rat hippocampus M1 receptors, and differed from those of rat pancreas and heart muscarinic receptors. The remaining (20%) muscarinic receptors showed low affinity for pirenzepine and AF-DX 116, being therefore of an M2β (or B) subtype, and were similar to rat pancreatic receptors.

Muscarinic receptor; Receptor binding assay; Receptor subtype; Neuroblastoma; (NB-OK cell, Human, Rat pancreas, Rat hippocampus)

1. INTRODUCTION

The NB-OK 1 cell line is a human neuroblastoma synthesizing and secreting the neuropeptide vasoactive intestinal polypeptide (VIP) and its precursor [1]. Other neuroblastoma cell lines, known to possess muscarinic receptors [2,3], provide interesting models to investigate the regulation of receptor synthesis, degradation and biological activity (see e.g. [2–6]). We therefore decided to test whether the NB-OK 1 cell line, like other neuroblastomas, expressed enough muscarinic receptors to allow binding studies.

Since at least three subtypes of muscarinic receptors have been described in mammalian tissues, we compared the binding properties of two selective ligands (pirenzepine and AF-DX 116) in order to identify the subtype(s) present in NB-OK 1 cells. Indeed, pirenzepine recognizes 'M1' or 'A' receptors with high affinity [7]; AF-DX 116 has a high affinity for M2α (or C) receptors [8], and M2β (or B) receptors show a low affinity for the two selective antagonists [8]. Finally, we compared the antagonist binding properties of these human receptors with those of similar M1 receptors, labeled by [^{3}H]pirenzepine, in rat hippocampus homogenates [9].

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2. MATERIALS AND METHODS

The NB-OK 1 cell line was a generous gift from Dr N. Yanaihara (Shizuoka College of Pharmacy, Shizuoka, Japan). *N*-[^{3}H]Methylscopolamine (70 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks, England) and

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[^{3}H]pirenzepine (80 Ci/mmol) from New England Nuclear (Dreieich, FRG). Pirenzepine was a generous gift from Dr R. Hammer (Institute de Angeli, Milano, Italy), 4-DAMP methobromide from Dr R. Barlow (University of Bristol, England), and hexahydrosiladifenidol (HHSiD) from Dr G. Lambrecht (Frankfurt am Main, FRG). We thank Dr A. Giachetti for helping us to obtain AF-DX 116 from Boehringer Ingelheim (FRG). Dexetimide and dicyclomine were generous gifts from, respectively, Janssen Pharmaceutica (Beerse, Belgium) and Merell-Dow (Brussels, Belgium). Trihexyphenidyl and atropine were obtained from Sigma (St Louis, MO, USA). All cell culture products were from Gibco (Ghent, Belgium).

The NB-OK 1 cell line was cultured as described in [1]. For studying the binding properties of muscarinic receptors, cells were collected in phosphate buffered (pH 7.4) saline (20 mM sodium phosphate, 150 mM sodium chloride) enriched with 1 mM EDTA; homogenized in 20 mM Tris-HCl buffer (pH 7.5) enriched with 5 mM $MgCl_2$, at a final concentration of 15 mg protein/ml, and stored in liquid nitrogen.

Hippocampus homogenates were obtained as described in [9].

In binding studies, we used with NB-OK 1 homogenates 800 μg protein and [^{3}H]NMS, and with hippocampus homogenates either 40 μg protein and [^{3}H]NMS or 200 μg protein and [^{3}H]pirenzepine. All homogenates were incubated for 2 h at 25°C, in 1.2 ml of 50 mM sodium phosphate buffer (pH 7.4) enriched with 2 mM $MgCl_2$ and 1% bovine serum albumin. Atropine (1 μM) was used to define nonspecific binding. The incubation was terminated by filtration, as described in [9].

3. RESULTS AND DISCUSSION

3.1. *Binding kinetics*

[^{3}H]NMS binding to NB-OK 1 cell homogenates was concentration- and time-dependent, as shown in fig.1 (left panel). [^{3}H]NMS dissociation was biphasic, with a half-life of 12 min at 25°C. These dissociation kinetics could be fitted by assuming that 80% of [^{3}H]NMS-receptor complexes had a k_{off} of 0.10 min^{-1}, typical of M1 receptors, and 20% a k_{off} of 0.02 min^{-1}, typical of M2β receptors (cf. [9]).

3.2. *Equilibrium binding*

[^{3}H]NMS saturation curves yielded a linear

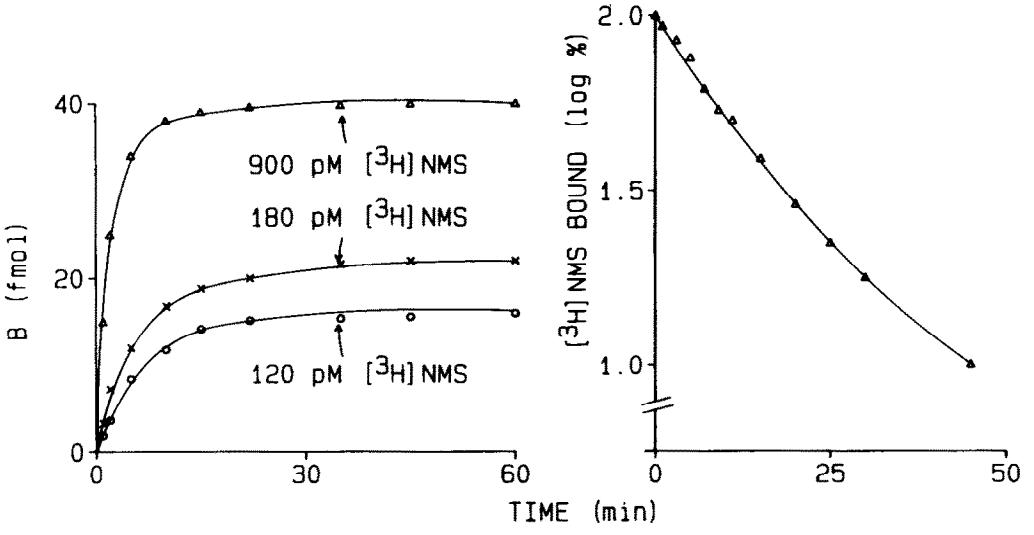

Fig.1. (Left) Specific binding of [^{3}H]NMS at three different concentrations (120 pM, ○; 180 pM, ×; 900 pM, △) to NB-OK 1 homogenates measured as a function of time. (Right) [^{3}H]NMS was preincubated with NB-OK 1 homogenates for 2 h before isotopic dilution. Residual specific binding was measured as a function of time. This experiment is representative of 2 others.

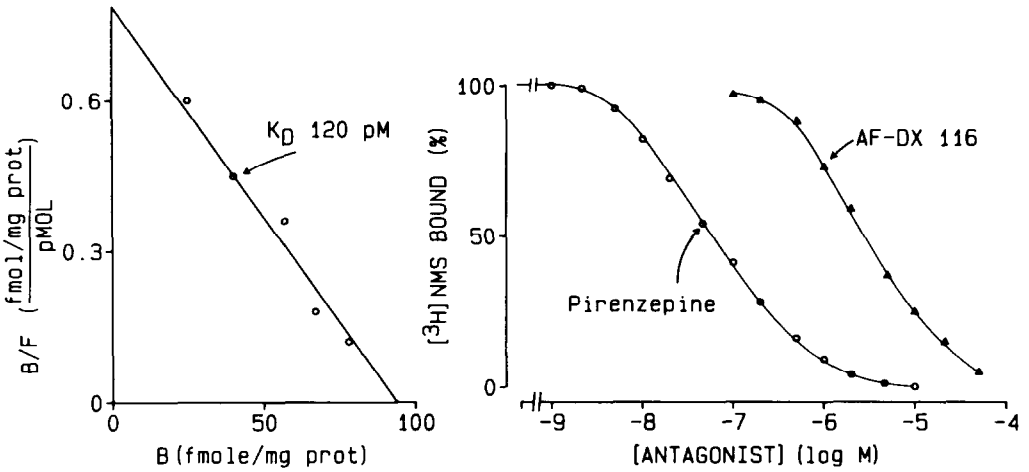

Fig.2. (Left) 60–1000 pM [^{3}H]NMS specific binding was measured at equilibrium. The results are presented according to Scatchard [10]. (Right) Specific [^{3}H]NMS (800 pM) binding was measured in the absence or presence of the indicated concentrations of pirenzepine (○) or AF-DX 116 (▲). This experiment is representative of 9 others.

Scatchard plot [10]. The K_d value was 120 pM, and maximal binding varied between 50 and 100 fmol/mg neuroblastoma cell lines [2–6].

Competition curves by the two selective antagonists pirenzepine and AF-DX 116, analyzed as in [9], indicated that 80% of the receptors had a high affinity for pirenzepine (K_i 5 nM), i.e. were of the M1 type, the remaining 20% showing a low affinity (K_i 140 nM), i.e. being of the M2 type (fig.2, right panel). All receptors had a low affinity for AF-DX 116 (K_i 500 nM). The NB-OK 1 receptors

Table 1

Dissociation constants (K_i value in nM) of 8 muscarinic antagonists for NB-OK 1 cell receptors labeled by [^{3}H]NMS, rat brain M1 receptors labeled by [^{3}H]pirenzepine, and rat heart and rat pancreas receptors labeled by [^{3}H]NMS (as in [11])

	NB-OK 1	Brain M1	Heart	Pancreas
Atropine	0.3	0.27	1.0	0.4
Pirenzepine	7	5	350	140
Dexetimide	0.15	0.14	1.50	0.2
Dicyclomine	3.0	3.0	56.0	3.0
Trihexyphenidyl	6.0	5.0	36.0	14.0
4-DAMP	0.9	0.9	9.0	1.2
HHSiD	16	16	200	17
AF-DX 116	500	500	50	1600

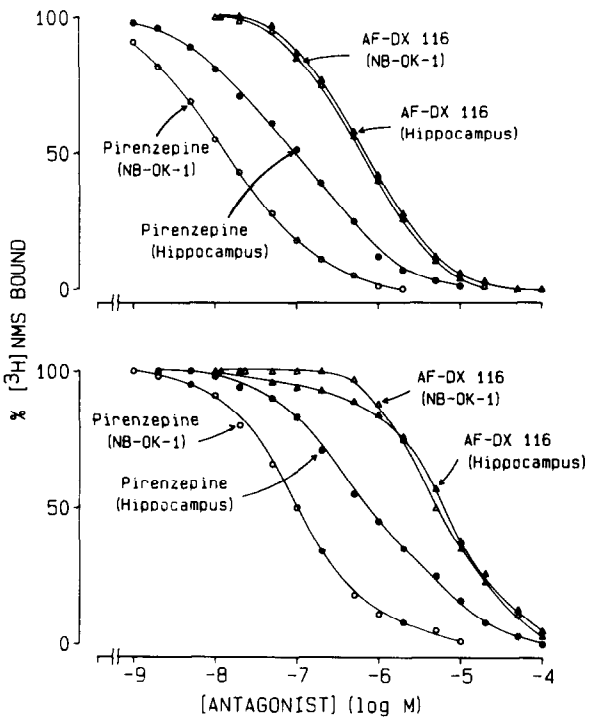

Fig.3. [^{3}H]NMS binding to NB-OK 1 (open symbols) and hippocampus (closed symbols) was measured in the absence or presence of pirenzepine (circles) or AF-DX 116 (triangles) at a 100 pM (top panel) or 1600 pM (bottom panel) total tracer concentration. This experiment is representative of 3 others.

consisted, therefore, of a majority of M1 and a minority of M2β receptors.

We next compared the binding properties of NB-OK 1 cell receptors with those of rat hippocampus M1 receptors labeled by [^{3}H]pirenzepine [9], and those of rat receptors labeled by [^{3}H]NMS in heart (M2α or C-like) and pancreas (M2β or B-like) homogenates (as in [11]). The 8 antagonists studied presented the same affinity towards NB-OK 1 muscarinic receptors and the subclass of hippocampus receptors labeled by [^{3}H]pirenzepine; the selectivity pattern was clearly different in rat heart and rat pancreas (table 1).

The proportion of M1 receptors found in NB-OK 1 cells was unusually large: by comparison, we found 55% of M1 receptors, 20% of C receptors and 25% of B receptors [9] in rat hippocampus (fig.3).

4. CONCLUSION

The NB-OK 1 human neuroblastoma expressed muscarinic cholinergic receptors showing nearly homogeneous binding properties, as 80% of these receptors had M1 receptor binding properties. The binding pattern of 8 muscarinic antagonists to these NB-OK 1 receptors differed from that in heart and pancreas muscarinic receptors and was identical to the binding pattern of receptors labeled by [^{3}H]pirenzepine in various rat brain regions [9].

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