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# Imidazopyridines as a Tool for the Characterization of Benzodiazepine Receptors: A Proposal for a Pharmacological Classification as Omega Receptor Subtypes

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LANGER, S. Z. AND S. ARBILLA. *Imidazopyridines as a tool for the characterization of benzodiazepine receptors: A proposal for a pharmacological classification as omega receptor subtypes.* PHARMACOL BIOCHEM BEHAV **29**(4) 763–766, 1988.—At present, the nomenclature of benzodiazepine (BZ) receptors is based on its historical association with the BZ structure. However, it is mainly through the new compounds chemically unrelated to BZs that the central and peripheral subtypes of BZ receptors have been characterized. We therefore propose the nomenclature of a greek letter omega, as $\omega_1$, $\omega_2$ and $\omega_3$ to designate respectively the central $BZ_1$, $BZ_2$ and the peripheral BZ receptor. Among the several classes of non-BZ drugs with affinity for different receptors, the imidazopyridines provide a valuable tool for the characterization of omega receptor subtypes. Most BZs are non selective ligands for the central $\omega_1$ and $\omega_2$ receptors while the selectivity for $\omega_1$ receptor subtypes is present in several non BZ chemical series: imidazopyridines (zolpidem), triazolopyridazines (CL 218872), betacarbolines ($\beta$-CCE) and pyrazoloquinolines (CGS 8216). Selective ligands for the $\omega_2$ subtype are not available so far. The so called peripheral BZ receptor is also present in the central nervous system, therefore the proposed nomenclature of $\omega_3$ receptors resolves this paradox because it does not designate location and it is defined in terms of pharmacological specificity. Selective ligands for $\omega_3$ receptors include the BZ Ro 5-4864, and the isoquinolinecarboxamide PK 11195, while the imidazopyridine alpidem is the ligand with the highest affinity for this receptor subtype.

Imidazopyridines Benzodiazepine receptors Omega receptor subtypes

THE criteria for the nomenclature and classification of pharmacological receptor subtypes are usually linked to the natural neurotransmitter and to the availablity of selective antagonist drugs. The recognition sites for benzodiazepines [9,13] however, are historically associated with this chemical class and, in spite of several proposals there are at present no established endogenous ligands acting either on the central or peripheral types of receptors. Moreover, several non-benzodiazepine compounds with high affinity for these central [2, 4, 7] and peripheral [6] receptors have been recently identified. The pronounced differences among the chemical structures of these exogenous ligands as well as the lack of definitive identification of endogenous ligands triggers the need for a pharmacological classification of receptors subtypes which is not bound as in the past to the benzodiazepine structure. Several chemical classes of non-benzodiazepines possess high selectivity for central benzodiazepine receptor subtypes ($BZ_1/BZ_2$) or for the peripheral type. Among them, a new chemical class, the imidazopyridines provide a valuable tool for the characterization of these receptor subtypes. Based on the inadequacy of the benzodiazepine nomenclature and the complexities associated with receptor localization, we propose the use of the greek letter omega to designate the three receptor subtypes as $\omega_1$, $\omega_2$ and $\omega_3$.

## METHOD

Rat cerebral cortex was homogenized for 60 sec using a Polytron (Ultra Turrax setting 50%) in 50 volumes of ice-cold buffer (50 mM Tris HCl, pH 7.4, 120 mM NaCl, 5 mM KCl), and centrifuged for 15 min at 50,000×g. The pellet was washed twice by rehomogenization and centrifugation, and

TABLE 1

INHIBITION BY NON-BENZODIAZEPINE LIGANDS AND FLUNITRAZEPAN OF THE BINDING OF $^3$H-BENZODIAZEPINES TO CENTRAL AND PERIPHERAL RECOGNITION SITES

| | Ki (nM) | | $IC_{50}$ (nM) |
|---|---|---|---|
| | $^3$H-Diaz Cerebellum | $^3$H-Diaz Spinal Cord | $^3$H-Ro 5-4864 Kidney |
| Zolpidem | 26 | 180 | 1,900 |
| Alpidem | 7 | 303 | 2 |
| CL 218872 | 390 | 1500 | >10,000 |
| Flunitrazepam | 2.5 | 1.4 | 430 |
| PK 11195 | N.T. | N.T. | 10.0 |

Drug concentrations inhibiting binding by 50% were obtained from inhibition curves derived from at least 8 concentrations of each drug tested. Values are mean of at least 2 independent experiments carried out in duplicate as described in [1].

finally resuspended in 100 volumes of buffer. The binding of $^3$H-zolpidem (S.A.: 60.5 Ci/mmol, LERS Chemistry Department) was determined by incubating at 0°C during 20 min 2.2 ml aliquots of the final membrane suspension with 0.1–30 nM [$^3$H]-zolpidem and other drugs in a final volume of 2.5 ml. At the end of the incubation period, two 1 ml samples were filtered under vacuum, through Whatman GF/B filters and immediately washed twice with 10 ml of ice cold buffer. Specific $^3$H-zolpidem binding was determined in the presence of 2 $\mu$M Ro 15-1788.

Rats' kidneys were decapsulated and homogenized for 60 sec in 100 volumes of chloride ion-free buffer ($Na^+/K^+$ phosphate 50 mM, pH 7.5) and filtered through four layers of sterile gauze. Aliquots of 0.1 ml of the final membrane suspension were incubated at 25°C for 120 min with $^3$H-alpidem (0.003–3 nM) (S.A.: 51.8 Ci/mmol, LERS Chemistry Department) and other drugs in a final volume of 2 ml. At the end of the incubation, two 0.9 ml samples were filtered under vaccum through Whatman GF/B filters previously treated with 0.05% polyethylenimine and immediately washed three times with 5 ml of ice cold buffer. Specific $^3$H-alpidem binding was defined in the presence of 1 $\mu$M Ro 5-4864.

## RESULTS

Table 1 shows the relative potencies and the selectivity of several non-benzodiazepine drugs for different receptor subtypes. The imidazopyridine zolpidem (Fig. 1) possesses preferential affinity for the $^3$H-diazepam binding site in the cerebellar cortex when compared with the spinal cord (Table 1). A similar profile was obtained with the triazolopyridazine CL 218872 (Table 1). The isoquinoline-carboxamide PK 11195 is very potent at the peripheral binding site labelled by $^3$H-Ro 5-4864 in membranes of the rat kidney (Table 1) while the imidazopyridine alpidem (Fig. 1) possesses a high selectivity for this peripheral site. The benzodiazepine flunitrazepam showed no selectivity between the sites labelled by $^3$H-diazepam in the cerebellum or in the spinal cord, while it was also active although less potent, at the level of the peripheral site labelled with $^3$H-Ro 5-4864 in the kidney (Table 1).

The saturation isotherm and Scatchard analysis of $^3$H-zolpidem binding to membranes of the cerebral cortex indicates that the high affinity binding of $^3$H-zolpidem corresponds to a single population of non interacting sites. The pharmacological profile of the $^3$H-zolpidem recognition site, which we propose to refer to as $\omega_1$, has the pharmacological properties of the benzodiazepine receptor of subtype$_1$ ($BZ_1$) [1]. Table 2 illustrates the affinity and density of $^3$H-zolpidem recognition sites in the rat cortex. The binding of $^3$H-zolpidem is enhanced by GABA to the same extent as that of $^3$H-diazepam [1].

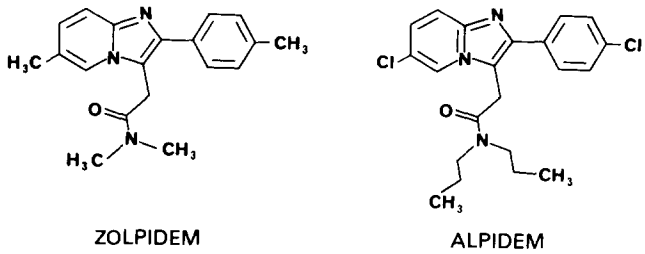


FIG. 1. Chemical structure of imidazopyridines.

Radiolabeled Ro 5-4864 and PK 11195 have by now been extensively used as ligands to characterize peripheral receptors (12–29). As shown in Table 2, the imidazopyridine $^3$H-alpidem also binds with high affinity in the rat kidney. The pharmacology of the recognition site labelled with $^3$H-alpidem in the rat kidney, which we propose to refer to as $\omega_3$ corresponds to that of the peripheral type of benzodiazepine receptor (data not shown).

## DISCUSSION AND THE PROPOSAL FOR A PHARMACOLOGICAL CLASSIFICATION

To avoid the confusion which arises from nomenclatures based on a single chemical series, on receptor localization or on receptor function, and in the absence of definitive proof for possible endogenous ligands, we propose a classification in which the greek letter omega ($\omega$) is employed to designate the three receptor subtypes.

The central $BZ_1$ receptor is designated as the $\omega_1$ receptor and corresponds to the central receptor identified by the selective agonists zolpidem, CGS 9896 and CL 218872 which include compounds from three different chemical series (Table 3) or by inverse agonists as the $\beta$-carboline $\beta$-CCE. Among the antagonists at $\omega_1$ receptors CGS 8216 appears to be a preferential receptor blocking drug, while flumazenil (Ro 15-1788) is not selective for $\omega_1$ receptors (Table 3). Examples of areas containing preferentially $\omega_1$ receptors are the molecular layer of the cerebellar cortex, the ventral pallidum and the substantia nigra [10].

The central $BZ_2$ receptor is designated as $\omega_2$ receptor and would correspond to the BZD receptor subtype that predominates for example in the dentate gyrus, and the caudate putamen [10]. Selective agonists or antagonists are not available for this receptor subtype. Both diazepam and flunitrazepam possess agonist properties on $\omega_2$ receptors although they also act on $\omega_1$ receptors. The antagonist flumazenil (Ro 15-1788) blocks nonselectively the $\omega_1$ and $\omega_2$ receptor subtypes (Table 3).

For the receptor which is referred to as peripheral BZ receptor and which is also present in the central nervous system, we propose the nomenclature of $\omega_3$. This receptor is present in peripheral tissues like kidney, platelets, pineal gland, adrenal cortex [5,8] and also in central structures of predominantly glial nature and in certain neuronal elements [3].

TABLE 2

SPECIFIC BINDING OF IMIDAZOPYRIDINES TO CENTRAL AND PERIPHERAL RECOGNITION SITES

| $^3$H-Imidazopyridine | Tissue | n | Kd (nM) | Bmax (fmol/mg prot) |
|---|---|---|---|---|
| $^3$H-Zolpidem | rat cerebral cortex | 5 | 7.10 ± 0.50 | 1700 ± 60 |
| $^3$H-Alpidem | rat kidney | 5 | 0.06 ± 0.01 | 2493 ± 150 |

The dissociation constant (Kd) and the maximal number of binding sites (Bmax) were calculated from linear Scatchard plots of saturation experiments by regression analysis. The data are means ± SEM of n individual determinations.

TABLE 3

PROPOSAL FOR A NEW NOMENCLATURE OF BENZODIAZEPINE RECEPTORS

| Receptor Nomenclature | | Selective Ligands | |
|---|---|---|---|
| Present | Proposed | Agonists or Inverse Agonists | Antagonists |
| Central $BZ_1$ | $\omega_1$ | Zolpidem [imidazopyridine]<br>CL 218872 [triazolopyridazine]<br>β-CCE [β-carboline] | CGS 8216 [pyrazoloquinolinone] |
| Central $BZ_2$ | $\omega_2$ | (—)* | (—)* |
| Peripheral $BZ_p$ | $\omega_3$ | Ro 5-4864 [benzodiazepine] | PK 11195 [isoquinoline-carboxamide] |

The current receptor nomenclature and the corresponding proposed pharmacological classification designated as omega receptor subtypes are presented. The corresponding chemical series for each compound is given between brackets.

(—)*: Selective ligands are not available.

The olfactory bulb and the spinal cord are areas which possess $\omega_3$ receptors in addition to $\omega_1$ or $\omega_2$ receptors. Alpidem is the imidazopyridine with the highest affinity for this $\omega_3$ site [8,12] while among the benzodiazepines, Ro 5-4864 is a selective ligand. The compound PK 11195 may possess antagonist properties at $\omega_3$ receptors. One of the questions which is still open is if the concept of inverse agonists, namely, drugs with negative efficacy at the level of $\omega_1$ receptors, can be extended to the $\omega_3$ receptor subtype.

The $\omega$ receptor subclassification provides the basis for the pharmacological characterization of the three receptor subtypes. Several of the therapeutic actions and the side effects in clinical use may be associated with selective interactions with the $\omega$ receptor subtypes. Therefore the availability of selective and specific receptor antagonists is essential in establishing the association between omega receptor subtypes and the central pharmacological effects: anxiolytic, anticonvulsant, myorelaxant and hypnotic.

At the level of the peripheral $\omega_3$ mediated effects, several possibilities are being explored: immunomodulation, control of cell proliferation and endocrine effects [11]. The development and characterization of $\omega_3$ receptor agonist and antagonists is also essential in the definition of the effects associated with the activation of these receptors.

A pharmacological classification of receptor subtypes offers the advantage of avoiding the confusion arising from a nomenclature based on a single chemical class of compounds or from the anatomical location as criteria for receptor characterization. The nature of the receptor mediated responses is further complicated because of the existence of classical agonist as well as inverse agonist drugs. Therefore, the proposed pharmacological classification of benzodiazepine receptor subtypes may open the way to their characterization through selective and specific drugs acting as agonists or antagonists on each of the three receptor subtypes which are designated by the greek letter $\omega$.

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