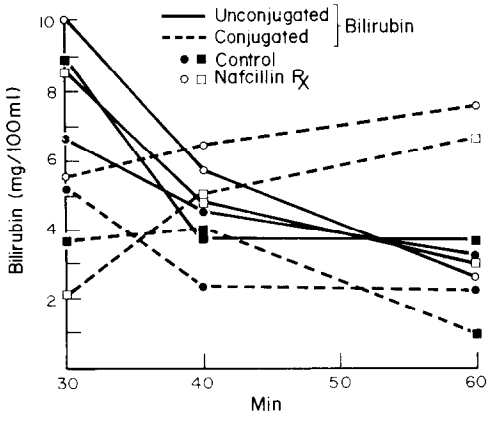

Fig. 3. Effects of nafcillin on plasma bilirubin disappearance rate. Each of two rats (circle and square) received bilirubin as two, 10 mg single intravenous boluses given 24 hr apart. Nafcillin (100 mg) was given on one occasion 30 min following a bilirubin injection.

Division of Hepatic Diseases
New York Hospital-Cornell Medical Center
New York, NY 10021, U.S.A.

CATHERINE ENG*
NORMAN B. JAVITT†

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* Post doctorate fellow AM 07142, Cornell University Medical College.

† Address all correspondence to: Dr. Norman B. Javitt, Division of Hepatic Diseases, New York University Medical Center, 550 First Ave., New York, NY 10016.

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Differences between cyclopyrrolones (suriclone and zopiclone) and benzodiazepines binding to rat hippocampus photolabelled membranes

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Since the discovery of benzodiazepine (BZD) receptors, it has generally been accepted that pharmacological actions of BZD are mediated through their interaction with their receptors. Some other drugs such as the two cyclopyrrolone derivatives, zopiclone (ZPC) and suriclone (SRC) which possess the same pharmacological profile but are chemically unrelated to BZD, have more recently been shown to have probably a similar mode of action [1, 2]. Moreover Ro 15-1788, a BZD derivative which antagonizes the central effect of classical BZD drugs has been revealed as a very interesting tool for studying interactions of BZD with their receptors. Indeed the nature of interaction is probably quite different in the case of BZD agonists on one hand and Ro 15-1788 on the other: in contrast to agonists, the affinity of the antagonist is neither increased in the presence of GABA nor decreased after photolabelling membranes with flunitrazepam (FLU) [3, 4]. It has, therefore, been assumed that the binding of the antagonist is not influenced by the conformational changes induced by GABA or photolabelling. Similar properties have been found for BZD antagonists of other chemical families such as β-carboline derivatives or the pyrazoloquinolinone CGS 8216 [5, 7]. Consequently it has been postulated that such differences could permit the distinction *in vitro* between agonists and antagonists.

However, Brown [8] recently found that the affinity of the two pyrazoloquinolinones CGS 9896 and CGS 9895, which have BZD agonist and partial agonist properties respectively [9], is not modified after photolabelling. These latter results indicate that affinity changes do not predict reliably pharmacological activity. With these conflicting results at hand it was particularly interesting to examine if the hypothesis linking agonist properties and large changes in affinity after photolabelling could nevertheless be validated using agonist drugs such as ZPC and SRC of a quite new and original chemical family.

Methods and results

Rat hippocampus membranes prepared as previously described [2] were labelled with non-radioactive FLU (3 nM, 10 mn, UV) and thereafter extensively washed (3 times) by centrifugation. in control experiments we made

Table 1. Specific binding of different [^{3}H] ligands in photolabelled and control rat hippocampus membranes

[^{3}H] ligand	Photolabelled membranes K_D (nM)	Photolabelled membranes B_{MAX} (%) (fmol/mg protein)	Control membranes K_D (nM)	Control membranes B_{MAX} (fmol/mg protein)
[^{3}H] FLU	1.30 ± 0.08	153 ± 6 (17)	1.30 ± 0.08	875 ± 34 (100)
[^{3}H] Ro 15-1788	0.98 ± 0.21	846 ± 36 (91)	0.85 ± 0.34	928 ± 6 (100)
[^{3}H] SRC	0.36 ± 0.09	697 ± 89 (89)	0.53 ± 0.17	783 ± 151 (100)

Table 2. Inhibition of specific [^{3}H] SRC binding to photolabelled and control rat hippocampus membranes. Results expressed by IC_{50} values (nM) are means ± S.E.M. of at least three different experiments

	Photolabelled membranes	Control membranes	IC_{50} ratio (photoshift)
Benzodiazepines			
Diazepam	2422 ± 284	34 ± 4	71
Flunitrazepam	209 ± 40	13 ± 2	16
Nitrazepam	752*	46*	16
Bromazepam	2414*	87*	28
Ro 15-1788	2.6 ± 0.5	1.8 ± 0.2	1.4
Cyclopyrrolones			
Suriclone	3.5 ± 0.5	2 ± 0.3	1.8
Zopiclone	243 ± 29	178 ± 46	1.4

* For these values only 2 experiments.

Table 3. Inhibition of specific [^{3}H] Ro 15-1788 binding to photolabelled and control rat hippocampus membranes. Results expressed by IC_{50} values (nM) are means ± S.E.M. of at least three different experiments

	Photolabelled membranes	Control membranes	IC_{50} ratio (photoshift)
Benzodiazepines			
Flunitrazepam	157*	5.9*	27
Ro 15-1788	1.9*	1.5*	1.3
Cyclopyrrolones			
Suriclone	2.1 ± 0.2	1.6 ± 0.1	1.3
Zopiclone	173 ± 5	67 ± 2	2.6

* For these values only 2 experiments.

sure that maximal photolabelling was achieved.

The specific binding of [^{3}H]FLU (86.1 Ci/mmole, New England Nuclear) [^{3}H] Ro 15-1788 (87.0 Ci/mmole, new England Nuclear) and [^{3}H] SRC (21.0 Ci/mmole, prepared by Mr. Pacot, Rhône-Poulenc and C.E.A.) were determined in control (U.V. light only) and photolabelled membranes. For all [^{3}H] ligands, non-specific binding was determined with 10 μM Ro 15-1788. The assays were performed at 0° in 50 mM Tris–HCl buffer pH 7.4. The incubations (2 hr) were terminated by filtration through Whatman GF/B glass fiber filters.

Under our conditions, specific [^{3}H] FLU binding is reduced by 83% after photolabelling. The K_D of [^{3}H] FLU binding is unchanged, only the B_{MAX} is decreased (Table 1). In contrast, the binding of [^{3}H] Ro 15-1788 and [^{3}H] SRC were unaffected by photolabelling. For both ligands, binding decreased only 9–11% without change in the K_D (Table 1). It seems that, after photolabelling, sites which have lost their ability to recognize FLU are still recognizing Ro 15-1788 and SRC. To investigate further these differences, we have compared the affinities of various drugs for BZD receptors using [^{3}H] SRC and [^{3}H] Ro 15-1788 binding in control and photolabelled membranes.

Using [^{3}H] SRC as ligand, the affinity of BZD agonists such as FLU or diazepam is strongly decreased after photolabelling (IC_{50} ratios higher than 10). In contrast, the two cyclopyrrolones, SRC and ZPC, and the BZD antagonist Ro 15-1788 have the same high affinities in the two types of membranes preparations (IC_{50} ratios close to 1.5) (Table 2). With Ro 15-1788 as radioligand, we obtain comparable results: a high IC_{50} ratio of 27 for FLU and IC_{50} ratios between 2.6 and 1.3 for ZPC, SRC and Ro 15-1788 (Table 3).

Discussion

Firstly, we demonstrate that specific binding of both the cyclopyrrolone agonist [^{3}H] SRC and the BZD antagonist [^{3}H] Ro 15-1788 are little affected by photolabelling (Table 1). Our results with [^{3}H] Ro 15-1788 are very similar to previous findings of Mohler and Gee [4, 6] with the same radioligand.

Secondly, using either ligand, we demonstrate a clear

difference in 'photoshifts' between the two cyclopyrrolones binding and BZD agonists binding. If we confirm that BZD agonists exhibit a high affinity shift in photolabelling membranes, in our hands the two cyclopyrrolones agonists have a low 'photoshift' (Tables 2 and 3). Low photoshifts for ZPC were also found by Möhler and Karobath [4, 5].

Our results clearly suggest that, contrarily to a recent hypothesis, the existence of a pharmacological agonist activity is not necessarily linked to a reduced affinity for photolabelled membranes. It must indeed be stressed that ZPC and SRC behave pharmacologically and clinically as agonists [10, 11]. It should also be noted that these findings with the cyclopyrrolone family agree well with the results reported by Brown [8] for the pyrazoloquinolinones.

Moreover, we confirm our previous data [2], indicating the existence of differences in the recognition of BZD agonists and SRC by BZD receptors. The BZD receptor model of Crippen [12] offers a tentative interpretation of these differences. According to this model, a ligand interacts with the receptor through 'binding site points' computed from structural and energetical data characterizing various agents able to bind to the BZD receptor site. In this model, ZPC interacts with less than half the number of binding site points associated with diazepam or FLU. This suggests that ZPC possesses a higher flexibility for binding to different conformations of BZD receptors induced by photolabelling.

In summary, we find that, in contrast to BZD agonists but like pyrazoloquinolinones, the two cyclopyrrolones agonists SRC and ZPC have a low photoshift whatever [^{3}H] ligand of BZD receptors is used.

These results raise a question about the possibility of generalizing beyond the BZD family the recent hypothesis linking a pharmacological agonist activity and a reduced affinity for photolabelled membranes.

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Rhône-Poulenc Recherches
Centre de Recherches de Vitry
13 quai Jules Guesde
F 94407 Vitry sur Seine
Cedex, France

J. C. BLANCHARD
J. L. ZUNDEL
L. JULOU

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Characterization of the amine oxidase activities of liver microsomes of different vertebrate and invertebrate species

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A clorgyline-resistant amine oxidase has been described in different tissues of several mammals [1–9]. This enzyme shows some similarities to the benzylamine oxidase activity which has been described in the blood plasma of non ruminant mammals [Blaschko, 10]. It clearly differs from the classical mitochondrial monoamine oxidase (MAO) (E.C. 1.4.3.4.) in its sensitivity to the inhibitors such as clorgyline and deprenyl [Buffoni, 11] and it is very similar, in its sensitivity to the inhibitors, to the diamine oxidase (DAO) (E.C.1.4.3.6.). Discrimination between clorgyline-resistant amine oxidase and DAO is possible only by means of competition experiments with different substrates. Because the physiological significance of the clorgyline-resistant amine oxidase is still unknown, it appeared interesting to us to characterize the amine oxidase activties of liver or digestive gland microsomes in vertebrate and invertebrate species in order to better understand their distribution in nature.

Materials and methods

The maintenance conditions of rat (Wistar), quail (*Coturnix coturnix japonica*), trout (*Salmo gairdneri*) and mussel (*Mytilus galloprovincialis*) are reported in Table 1.

The animals were killed by decapitation or by a blow to the head. The liver was removed, weighed, then perfused with an ice-cold solution of KCl 0.15 M at pH 7.4 and homogenized in a glass–Teflon homogenizer at 1:4 (w/v) ratio with ice-cold sucrose 0.25 M containing tris [Tris(hydroxymethyl)amino-methan] 0.05 M at pH 7.4. The homogenates were centrifuged at 9000 *g* for 20 min; the resulting supernatant was centrifuged at 105,000 *g* for 60 min. The pellets were resuspended by hand in a glass–Teflon homogenizer in KCl 0·15 M at pH 7.4 and centrifuged at 105,000 *g* for 50 min; the final pellets were stored at −70° until the enzymatic assays (less than 2 weeks) were performed.