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THE INHIBITION OF MONOAMINE UPTAKE INTO RAT BRAIN SYNAPTOSOMES BY SELECTED BICYCLO-OCTANES AND AN ANALOGOUS BICYCLO-OCTENE

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Abstract—The ability of a cis bicyclo-octane (LR5182), its optical isomers (LR5866) and (LR5953), a trans bicyclo-octane LR5309 and a bicyclo-octane LR5659 to inhibit the uptake of noradrenaline, dopamine and 5-hydroxy tryptamine was studied in an *in vitro* synaptosomal preparation from rat brain areas. The activity of the novel compounds in the system was compared to that of known uptake inhibitors and anti-depressants. Only LR5309 showed significant activity against 5HT having an IC_{50} of 0.59 μM compared to that of imipramine of 0.44 μM. All the novel compounds were active at inhibiting noradrenaline and dopamine uptake. The most interesting compounds were LR5182 and its two optical isomers since these compounds had a similar IC_{50} to that of desipramine (DMI) in blocking noradrenaline uptake (DMI IC_{50} = 0.27 μM, 5182 IC_{50} = 0.11 μM) and were the most active compounds so far reported in blocking dopamine uptake (5182 IC_{50} = 0.069 μM, nomifensin IC_{50} = 0.23 μM). Since 5182 and its isomers are as active at blocking noradrenaline uptake as known antidepressants, and in addition are very potent at blocking dopamine uptake, these compounds may provide a useful treatment for certain categories of depressed patients.

A series of cis and trans bicyclo-octanes and an analogous series of bicyclo-octenes were assesssed for their potential anti-depressant activity using the reversal of hypothermia induced in rats by reserpine and their ability at a concentration of 10^{-6} M, to inhibit the uptake of noradrenaline and 5-hydroxytryptamine into a crude synaptosomal preparation from whole mouse brain. On the basis of this preliminary data the most active compound from each series was selected for further investigation.*

Figure 1 illustrates the structures of compounds LR5182 and LR5309, which were selected from the cis and trans bicyclo-octane series respectively and compound LR5659, which was selected from the bicyclo-octene series. Compound LR5182 was resolved into its two isomers, the l-form LR5953 and *d*-form LR5866. In this communication we report the ability of these compounds to inhibit the uptake of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5HT) and compare their activity with known uptake inhibitors.

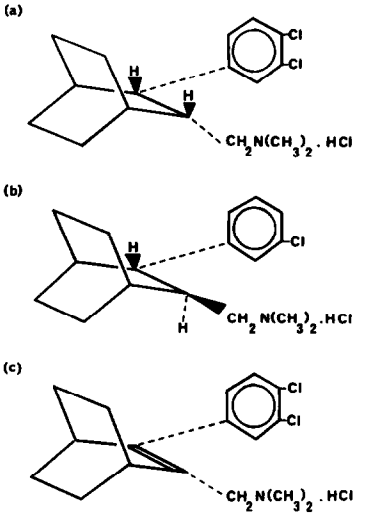

Fig. 1. Structures of (a) LR5182, 5953 and 5866 ClS-3-(3,4-dichlorophenyl)-2-*N*,*N* dimethylamino methyl-bicyclo-//2,2,2/-octane, hydrochloride (b) LR5309 Trans,-3-*m*-chlorophenyl-2-*N*,*N*'/-dimethylaminomethyl-bicyclo-/2,2,2/-octane hydrochloride (c) LR5659 3-(3,4-dichlorophenyl)-2-*N*,*N* dimethylaminomethyl bicyclo-/2,2,2/-oct-2-ene,hydrochloride.

MATERIALS AND METHODS

Female Lilly Wistar rats (200–250 g), fed and watered *ad libitum*, were killed by cervical dislocation and the brains immediately removed and placed on ice for dissection. After removal of the cerebellum, pineal body and olfactory lobes, the corpora striata were dissected out through the lateral ventricles. The hippocampus was discarded, the cortex dissected out and tissue below the rhinal fissure was removed. The remaining mid and hind brain areas were combined. The uptake of NA, DA and 5HT was measured in the cortex, corpus striatum and mid-hind brain respectively.

All experiments were performed on the pooled tissue from two rats which was homogenised by hand, in a glass homogeniser with a Teflon pestle (radial clearance 0.25–0.30 mM), in ice-cold 0.32 M sucrose containing 10 mM glucose. The corpora striata were homogenised in 48 vols (w/v) and the cortex and mid-hind brain each in 8 vols (w/v). The homogenates

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were centrifuged at 1000 *g* for 10 mins at room temperature, the supernatant removed and gently mixed before use. A 0.1 ml aliquot of the supernatant was added to 0.9 ml Krebs–Hensleit buffer, pH 7.4. The buffer, which had been pre-incubated for 5 min at 37° under O_2/CO_2(95/5 v/v), contained 5.3 mM glucose, 0.5 mM EDTA, 1.1 mM ascorbic acid, 12.5 μM nialamide, [^{14}C]-monoamine (5-hydroxy [side chain-2-^{14}C] tryptamine creatinine sulphate, 54–56 mCi/mmol, ethylamine-2-^{14}C dopamine hydrochloride, 50–60 mCi/mmol or DL-[methylene-^{14}C] noradrenaline-DL-bitartrate, 53–62 mCi/mmol from The Radiochemical Centre, Amersham) and where appropriate, test compound. The incubation was continued for a further 5 min with continued gassing and the reaction stopped by rapid centrifugation at 5600 *g* for 15 min at 0°C. The supernatant was discarded, and after surface washing at room temperature with 3 ml *n*-saline, the pellet was digested in 0.2 ml hydrogen peroxide (100 vols) for 30 min at room temperature. The digestate was transferred to a counting vial with 10 mls Bray's solution and the radioactivity determined.

For the determination of the affinity constants (Km) of the monoamines, the concentration of the relevant [^{14}C]-monoamine in the incubation medium was varied and no test compound was added. The uptake of the [^{14}C]monoamine at each concentration was determined in triplicate and the Km was calculated from a Lineweaver–Burk plot.

For the determination of the concentration of drug causing 50 per cent inhibition (IC_{50}) of the uptake of [^{14}C]-monoamines, the concentration of the relevant [^{14}C]-monoamine was 4.8×10^{-8} M, and at least five concentrations of the test compound were used. The final IC_{50} is a mean of at least three values, determined in independent experiments and in each experiment the percentage inhibition of the uptake of the [^{14}C]-monoamines, at the concentration of the test compound used, was determined in triplicate.

RESULTS AND DISCUSSION

The Kms for noradrenaline in the cortex, 5-hydroxytrypamine in the mid-hind brain and dopamine in the corpus striatum were 6×10^{-7} M, 1.9×10^{-7} M and 3.3×10^{-7} M respectively. These values are in close agreement with those previously reported in the literature for the high affinity uptake sites in the areas used [1, 2].

The IC_{50}'s of the uptake of the [^{14}C]-monoamines by our novel compounds and by known antidepressants are shown in Table 1. It can be seen that all the novel compounds are potential inhibitors of amine uptake. A comparison of the three chemical types, namely trans bicyclo-octane, cis bicyclo-octane and bicyclo-octene, represented by LR5309, LR5182 and LR5659 respectively, demonstrate three different profiles of activity. Compound LR5309 is the least active against NA and DA, but is the most active of these compounds in inhibiting 5HT uptake. This compound thus lacks specificity in the monoamine uptake system. Unlike LR5309, LR5659 and LR5182 are relatively inactive against 5HT uptake, but are active against NA and DA uptake. The difference between these two latter compounds lies in the greater potency of LR5659 against NA uptake. The two isomers of LR5182 (LR5953 and LR5866) have similar profiles to each other and to LR5182, the l-isomer 5953 being the most potent. The IC_{50} values obtained for LR5182 are very close to the mean of the IC_{50} values of its isomers.

The tricyclic antidepressants are thought to act by inhibiting the re-uptake of NA or 5-HT from the synaptic cleft, thus correcting a reduced transmission in one of these neuronal systems. In considering our novel compounds as potential antidepressants it is, therefore, relevant to compare their ability to block monoamine uptake with known antidepressants. Imipramine is a potent blocker of 5HT uptake (IC_{50} 0.44 μM) and clearly, with the exception of LR5309 (IC_{50} 0.59 μM), our compounds are less active in this system.

Two clinically active antidepressants, which are potent blockers of NA uptake are desipramine (IC_{50} 0.27 μM) and nomifensine (IC_{50} 0.041 μM). All our compounds have a potency similar to or greater than that of desipramine and the most active of these, LR5659 (IC_{50} 0.048 μM), is similar in activity to nomifensine. Clearly, if blockade of NA uptake is an indication of potential antidepressant activity, our compounds are comparable with the most potent of the known clinically effective antidepressants.

Table 1. Concentration of drug (μM) causing a 50 per cent inhibition (IC_{50}) of [^{14}C]–NA, [^{14}C]–5HT and [^{14}C]–DA uptake by synaptosomes prepared from specific areas of rat brain

Compound	[^{14}C]–NA cortex	[^{14}C]–5HT mid/hind brain	[^{14}C]–DA corpus striatum	IC_{50} Ratio NA/DA
LR5309	0.14 ± 0.08 (4)	0.59 ± 0.12 (3)	0.34 ± 0.18 (3)	0.41
LR5659	0.048 ± 0.022 (4)	1.1 ± 0.1 (3)	0.04 ± 0.019 (4)	1.04
LR5182	0.11 ± 0.03 (4)	3.3 ± 1.0 (7)	0.069 ± 0.021 (4)	1.59
LR5866	0.20 ± 0.13 (4)	8.7 ± 3.4 (3)	0.082 ± 0.039 (4)	2.44
LR5933	0.082 ± 0.002 (3)	1.2 ± 0.1 (3)	0.048 ± 0.014 (4)	1.71
Imipramine	6.4 ± 7.5 (5)	0.44 ± 0.08 (3)	11.0 ± 6.0 (3)	0.58
Desipramine	0.27 ± 0.03 (3)	3.2 ± 1.4 (3)	14.0 ± 4.0 (3)	0.02
Nomifensine	0.041 ± 0.005 (3)	20.0 ± 14.0 (3)	0.23 ± 0.06 (3)	0.18

The results are expressed as the mean ± standard deviation of independent IC_{50} determinations. The value in brackets is the number of determinations.

It is in the ability of our compounds to block the uptake of DA that their activity differs markedly from that of the tricyclic antidepressants, which have little activity in this system [3]. Nomifensine is the first clinically active antidepressant which is a potent inhibitor of DA uptake [4]. It has been reported that nomifensine is more potent than amphetamine or benztropine in the DA system and we have confirmed this in our system, obtaining IC_{50} values for amphetamine and benztropine of $0.42 \pm 0.10\ \mu M$ and $0.47 \pm 0.23\ \mu M$ respectively and an IC_{50} of $0.23 \pm 0.06\ \mu M$ for nomifensine. Our compounds LR5182, 5866, 5953 and 5659 are all more potent than nomifensine in blocking the uptake of DA. Significantly the ratio of activity in the NA and DA systems is markedly different for these four novel compounds than for the known antidepressants (Table 1), this being most marked for 5182 and its two isomers.

There is evidence that DA turnover is reduced in some forms of depression [5, 6, 7] and that the DA precursor, L-DOPA has an antidepressant effect in retarded depressed patients [8, 9]. A compound with the ability to increase DA transmission, such as LR5182 or its isomers, may therefore be particularly useful in these cases.

In summary we have examined the ability of four bicyclo-octanes and a bicyclo-octene to block the uptake of [^{14}C]-monoamines into rat brain synaptosomes and compared their potency with known antidepressants. All the novel compounds are active in this system, showing varying profiles of activity related to structure. The cis-bicyclo-octane and its isomers show a novel profile, being the most active blockers of DA uptake so far reported in the literature and having a greater ability to block DA uptake than NA uptake, although their potency in blocking NA uptake is similar to the known antidepressant, desipramine.

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