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Pharmacological profile of a chromanamine analogue (DP-6OH-3CA) of the selective presynaptic dopamine agonist *N,N*-dipropyl-7-hydroxy-2-aminotetralin

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Abstract—The pharmacological profile of an oxygen isostere of the selective presynaptic dopamine agonist DP-7OH-AT, i.e. dipropyl-6-hydroxy-3-chromanamine (DP-6OH-3CA) has been evaluated in various receptor binding, neurobiochemical and behavioural experiments. The chromanamine displaced the ^{3}H-labelled dopamine ligands, 5,6-DPAT and N-0437, with K_i values of 106 and 143 nM, respectively. In in-vivo biochemical models for presynaptic activity the chromanamine induced a half-maximal effect in the γ-butyrolactone reversal test at 6·8 μmol kg^{-1} and had an ED70 value of 40 μmol kg^{-1} for HVA decrease in the striatum. In behavioural models for postsynaptic dopaminergic activity a half-maximal effect for the induction of stereotypy was reached at 100 μmol kg^{-1} and reversal of the effects of reserpine to a level of 200 counts was induced at 11 μmol kg^{-1}. On comparison of these results with the results obtained with the carbon analogue of DP-6OH-3CA, i.e. DP-7OH-AT, it is apparent that the chromanamine has a reduced potency for dopamine D_2 receptors in in-vitro and in-vivo models. The selectivity for presynaptic dopamine receptors was lower than with DP-7OH-AT and the isomeric chromanamine, DP-8OH-3CA, indicating that the optimal position of the hydroxyl group for presynaptic selectivity is in the 8 and not in the 6 position for the chromanamines.

In our laboratory several dopamine (DA) agonists of the 2-aminotetralin class have been synthesized and, recently, oxygen isosteres of the aminotetralins, the chromanamines, have also been prepared (Horn et al 1984; Vermue et al 1986). In the latter study the oxygen isostere of *N,N*-dipropyl-5-hydroxy-2-aminotetralin (DP-5OH-AT), DP-8OH-3CA, was found to be a potent and selective D_2 agonist with enhanced selectivity for presynaptic DA receptors. Dopamine agonists inhibit the activity of dopaminergic neurons via presynaptic receptors by inhibition of tyrosine hydroxylase activity, dopamine release and firing frequency (Stoof & Kebabian 1984). It is possible that selective presynaptic agonists could be employed to suppress the dopaminergic system and might therefore be of clinical relevance (Meltzer 1980). Of the various 2-aminotetralins that have so far been investigated, *N,N*-dipropyl-7-hydroxy-2-aminotetralin (DP-7-OH-AT, Fig. 1A) has been shown to have the highest degree of autoreceptor selectivity (Mulder et al 1987). Bearing in mind the enhanced presynaptic selectivity found for DP-8OH-3CA we decided to synthesize an oxygen isostere of DP-7OH-AT in the hope that the autoreceptor activity might be further enhanced. We have therefore synthesized *N,N*-dipropyl-6-hydroxy-3-chromanamine (DP-6OH-3CA, Fig. 1A).

The purpose of the present study was to evaluate the pharmacological profile of DP-6OH-3CA in receptor binding, neurobiochemical and behavioural experiments.

Materials and methods

Animals. Male Wistar rats (180–230g, CDL Groningen) and male albino mice (20–30g, TNO Rijswijk) were used and they were housed in a 10·5 h light, 13·5 h dark cycle at 21°C. The animals had free access to food and drink. The experiments were performed between 0900 and 1800 h at 21°C in the light.

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Drugs. The drugs administered to rats were given in a solution of 2 mL kg^{-1}, the solutions injected into mice were given in a volume of 5 mL kg^{-1}. Most drugs were solubilized in saline, but reserpine was injected in a solution of benzyl alcohol (0·08 mL/10 mL), citric acid (10 mg/10 mL), polysorbate 80 (0·8 mL/10 mg) and water (The Extra Pharmacopoeia, 27th Ed., Martindale). The drugs were obtained from the following companies: reserpine (Brocacef), *m*-hydroxybenzylhydrazine-2HCl (NSD 1015; Janssen) and γ-butyrolactone (GBL; Aldrich). The 2-aminotetralin and 3-aminochromane analogues were synthesized in our own laboratory. Elemental analysis showed that the values were within ±0·4% of the theoretical ones.

The mice that were pretreated with reserpine, had been injected i.p. 16–18 h before the experiment with a dose of 8·2 μmol kg^{-1} and were found to be immobile and felt cold to the touch (23 to 28°C). The neurochemical measurements in brain samples are expressed as percentages of wet weight together with s.e.m. Tests for the statistical significance of the results were performed with the Student's *t*-test ($P < 0.05$).

Receptor binding. To test the ability of DP-6OH-3CA and DP-7OH-AT to displace a dopaminergic agonist from its receptor, the displacement of [^{3}H] 2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin ([^{3}H] N-0437) and [^{3}H] *N,N*-dipropyl-5,6-dihydroxy-2-aminotetralin ([^{3}H] 5,6 DPAT) to high affinity D_2 receptors in striatal membranes of calf and rat, respectively, was investigated in sodium-free buffer. These labels have been shown to bind with high affinity to D_2 receptors (Mulder et al 1985; Van der Weide et al 1987). [^{3}H]N-0437 had a specific activity of 80·6 Ci mmol^{-1} (Amersham, UK), it had a K_D value of 0·17 nM and was used in the competition experiments at a concentration of 1·5 nM. Inhibition of the ligand binding was determined in triplicate using 11 concentrations of the test compound (up to a concentration of 10^{-5} M). The specific binding was defined as the difference in the amount of radioactivity bound in the absence or presence of 1 μM unlabelled N-0437. The calf caudates were homogenized and washed according to the method of Van der Weide et al (1987) resulting in a final tissue content of 2·5 mgL^{-1}. The ^{3}H-labelled ligand 5,6 DPAT had a specific activity of 229 Ci mmol^{-1} (Amersham, UK), a K_D value of 0·57 nM and was used at a concentration of 0·4 nM. The specific binding of this ligand was determined using 1 μM (+)-butaclamol as the specific displacer. The [^{3}H]DPAT binding was studied using rat caudate membranes and was in other aspects similar to the [^{3}H]N-0437 binding experiments. The K_i values were obtained from the competition curves, using a Hill plot.

Biochemical models: a. γ-Butyrolactone reversal. The γ-butyrolactone (GBL) experiments were performed according to the method of Walters & Roth (1974). DP-60H-3CA was injected i.p., subsequently followed by GBL (8·7 mmol kg^{-1} i.p.) and NSD 1015 (0·47 mmol kg^{-1} i.p.) at 5 mins intervals. Thirty minutes after the administration of GBL the rats were killed and the striata and tubercles dissected and frozen at −80°C. The dihydroxyphenylalanine (DOPA) levels were assayed as described above (Westerink & Mulder 1981).

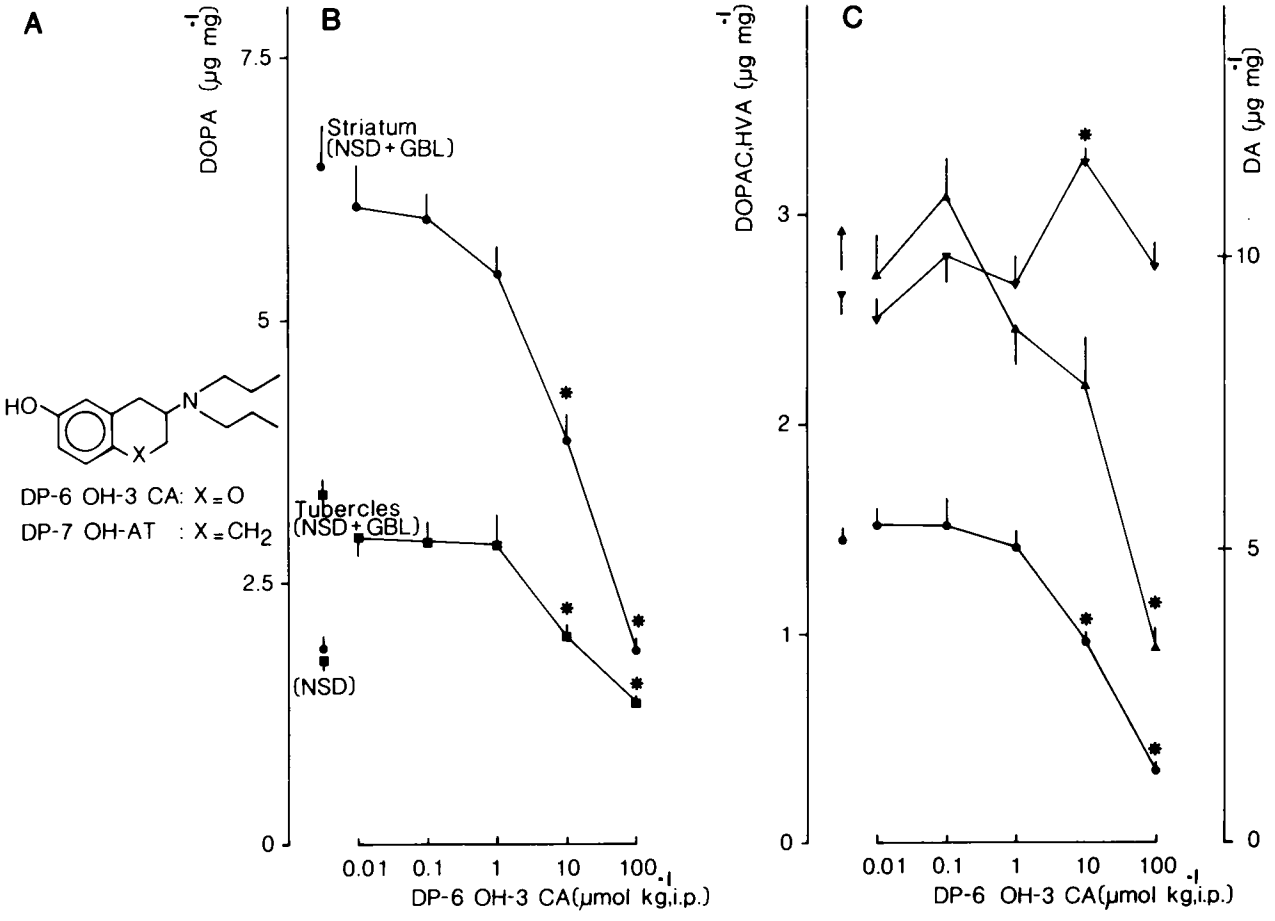

FIG. 1. A. Structures of DP-6OH-3CA and DP-7OH-AT. B. Dose-response curves of DP-6OH-3CA for the reversal of the GBL induced DOPA accumulation after inhibition of DOPA decarboxylase with NSD 1015 in striatum (●) and tubercles (■) of male rats (*: $P<0.05$, n = 6–8). C. Dose-response curves for the effect of DP-6OH-3CA (45 min before decapitation) on DOPAC (▲), HVA (●) and DA (▼) levels in striatum (wet weight, *: $P<0.05$, n = 6–8).

b. Dopamine metabolism. In order to study the influence of DP-6OH-3CA on dopamine metabolism, rats were injected i.p. with solutions of the drugs, placed in cages with food and water and were killed 30 mins later. This period was determined as the time necessary to obtain a maximal response with 30 μmol kg^{-1} of the test drug in pilot experiments. The animals were killed by cervical dislocation, the brains were removed and the samples were dissected onto dry ice and were stored at −80°C. Following weighing of the samples, homogenization in perchloric acid (0·1 M, 1 mL) and centrifugation (3000*g*, 5°C, 15 min), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed using high performance liquid chromatography (HPLC) combined with electrochemical detection following separation on G10 columns (Westerink & Mulder 1981). Cerebella were used as tissue blanks and no corrections were made for the recoveries (85–95%).

Behavioural models: a. Stereotypy. The rats were placed in cages and were allowed to acclimatize for 30 min and were subsequently injected i.p. and scored for their behaviour. Stereotyped behaviour was determined in a double blind way every 5 min using the four point ranking scale of Costall et al (1977) for sniffing and biting/licking with simultaneous recording of the observed behaviour on a check-list. The plateau levels of these individual time curves were used to calculate the average scores with s.e.m..

b. Locomotion. Locomotion was measured with Automex activity recorders and the counts were printed every 5 min. The reserpinized mice were injected i.p. with the test compound or saline and immediately placed in the cages (3 mice per cage). Hyperlocomotion with rats was determined simultaneously with the scoring of the stereotyped behaviour (one animal per cage). In both cases the total counts sampled during the 2 h following injection of the animals were used.

Results

Receptor binding. The ^{3}H-labelled D_2 agonist N-0437 was displaced by DP-6OH-3CA with a K_i value of 143 nM and by DP-7OH-AT with a K_i value of 6·5 nM. The [^{3}H]5,6 DPAT label was displaced with K_i values of 106 and 17·6 nM for DP-6OH-3CA and DP-7OH-AT, respectively.

Biochemical models: a γ-Butyrolactone experiments. The DOPA levels of NSD 1015 treated (control) samples were 1·75 ± 0·10 μg mg^{-1} (n = 8) in tubercles and 1·84 ± 0·17 μg mg^{-1} (n = 8) in striata. With GBL treatment these values increased to 3·34 ± 0·15 (n = 6) and 6·42 ± 0·39 μg mg^{-1} (n = 6), respectively. The chromanamine dose-dependently reversed the GBL induced DOPA accumulation in both brain areas (Fig. 1B). At a dose of 100 μmol kg^{-1} the chromanamine induced a DOPA reversal of 96% in striata and 134% in tubercles. The test

compound induced a 50% reversal at 6·8 μmol kg^{-1} in striata and at 2·3 μmol kg^{-1} in tubercles.

b. Dopamine metabolism. In saline-treated animals the DOPAC, HVA and DA levels in striata were 2·92±0·26 (n=8), 1·45±0·05 (n=8) and 9·34±0·30 (n=7) μg mg^{-1} wet weight, respectively; in tubercles these values were 2·17±0·26 (n=8), 0·52±0·03 (n=7) and 8·46±0·30 (n=7) μg mg^{-1} wet weight, respectively. The chromanamine dose-dependently decreased and DOPAC and HVA levels of the striata (Fig. 1C) and tubercles. A decrease of 30% was found at 40 μmol kg^{-1} in striata and at 15 μmol kg^{-1} in tubercles. The dopamine level was significantly increased at 10 μmol kg^{-1} in striata (Fig. 1), but not in the tubercles or at lower or higher doses in the striata.

Table 1. Behavioural effects of DP-6OH-3CA.

Stereotypy			
Dose	Score	N	*P*
10 μmol kg^{-1}	0·2±0·15	5	n.s.
30 μmol kg^{-1}	1·1±0·4	5	<0·05
100 μmol kg^{-1}	2·0±0·5	5	<0·05
Hyperlocomotion	Counts (2h)		
Saline	650±150		
10 μmol kg^{-1}	550±250	5	n.s.
30 μmol kg^{-1}	1000±150	5	n.s.
100 μmol kg^{-1}	3500±1000	5	<0·05
Reserpine reversal	Counts (2h)		
Saline	54±10	4	
10 μmol kg^{-1}	240±140	4	n.s.
30 μmol kg^{-1}	440±40	4	<0·05
100 μmol kg^{-1}	1280±120	4	<0·05
300 μmol kg^{-1}	1920±220	4	<0·05

The ability of DP-6OH-3CA to induce stereotypy in rats was measured using the 4-point ranking scale of Costall et al (1977). The hyperlocomotion was measured simultaneously with the stereotypy in rats.
The reserpine reversal experiments were measured in mice (3 mice per cage). The statistical significance of the results, compared with saline-treated animals, was determined with the Student's *t*-test (n.s. = not significant).
For further details see Methods.

Behavioural models: Stereotypy and hyperlocomotion. The chromanamine induced sniffing in rats at 30 and 100 μmol kg^{-1} (i.p., Table 1) reaching a half-maximal effect (constant sniffing) at 100 μmol kg^{-1}. With this dose the behavioural response reached its maximum after half an hour which was maintained for 1·3 h and then slowly declined.

Reserpine reversal. After reserpine pretreatment, the mice were immobile and felt cold to the touch. The chromanamine enhanced the locomotion of the mice at doses of 30 μmol kg^{-1} (i.p.) and higher (Table 1). At a dose of 100 μmol kg^{-1} the maximal effect was reached 15 min after the injection and it had declined to half this effect 25 min later. The mice exhibited stereotyped sniffing during the latter test.

Discussion

The displacement of the ^3H ligands N-0437 and 5,6 DPAT by DP-6OH-3CA and DP-7OH-AT show that the chromanamine is a compound active at high affinity D_2 receptors with an in-vitro potency that is between 6 and 22 times lower than its carbon analogue. The test compound was able to reverse the GBL induced increase in DOPA levels both in the striatum and in the olfactory tubercles inducing a half-maximal effect in the striatum at 6·8 μmol kg^{-1}. However, in earlier experiments in our laboratory (Van Oene et al 1984) it was found that DP-7OH-AT produced this effect at 0·41 μmol kg^{-1}, indicating that the in-vivo potency of DP-6OH-3CA at DA autoreceptors is decreased to a similar extent as was found in-vitro.

To estimate the effects of DP-6OH-3CA on DA metabolism the levels of DOPAC and HVA were measured in striata and tubercles and were found to be inhibited by 30% (ED70, Feenstra et al 1983) at 40 μmol kg^{-1} in the striatum. On comparison of this result with the effects of DP-7OH-AT measured earlier in our laboratory (Feenstra et al 1983), it is apparent that with this test a 29 times lower in-vivo potency is found. Hence DP-6OH-3CA has an in-vitro and in-vivo potency between 6 and 29 times lower than its carbon analogue. The oxygen isostere of DP-5OH-AT, DP-8OH-3CA, also showed a restricted in-vivo potency (Vermue et al 1986). However, this effect was thought to be caused by metabolic degradation, since the in-vitro potency was enhanced in comparison to its carbon analogue. Hence it can be concluded that oxygen substitution in DP-7OH-AT has yielded a compound with a similarly reduced potency for dopamine receptors both in-vivo and in-vitro.

The experiments on dopamine metabolism showed that the dopamine level was increased at 10 μmol kg^{-1} in the striatum whilst no effects were seen with the other doses, or in the tubercles. The inhibition of dopamine synthesis and release apparently had cancelled each others effects out at 45 min after the injection.

In the behavioural postsynaptic model i.e. induction of stereotypy, DP-6OH-3CA was effective at rather high doses: a half-maximal effect was obtained at 100 μmol kg^{-1}. In mice, reserpine reversal to a level of 200 counts (2 h) was found at 11 μmol kg^{-1}. When these figures are compared with the results obtained for DP-7OH-AT (Feenstra et al 1983; Van Oene et al 1984) they show that the chromanamine is 5 times less and 3 times more potent, respectively. The low potency found in the stereotypy test is in accordance with the results obtained with the presynaptic models. The selectivity for presynaptic dopamine receptors was also lower than with DP-7OH-AT; using GBL reversal and reserpine reversal as indices, a 51 times lower value was found and with GBL reversal and stereotypy as indices, a 5 times lower selectivity is apparent. Hence it can be concluded that the optimal position of the hydroxyl group for presynaptic dopamine selectivity with 2-aminotetralins (Van Oene et al 1984) is different for chromanamines since the DP-8OH-3CA analogue (Vermue et al 1986) has a much better selectivity in this regard than DP-6OH-3CA.

In summary, the oxygen isostere of DP-7OH-AT, DP-6OH-3CA, has a decreased in-vitro affinity for dopamine D_2 receptors which is also apparent in in-vivo models. The optimal position of the hydroxyl group in chromanamines for presynaptic dopaminergic selectivity seems to be at the 8 and not at the 6 position and in this respect there is a distinct difference in the structure activity relationships between the 2-aminotetralins and the 3-chromanamines.

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Frequency-dependent autoinhibition of histamine release from rat cortical slices: a possible role for H_3 receptor reserve

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Abstract—The inhibition of histamine release after depolarization of rat cerebral cortex slices by electrical stimulation and mediated by the postulated presynaptic autoreceptor (H_3) depends strongly on the conditions of stimulation. Using electrically stimulated slices of the cortex a rightwards shift of the concentration-response curve of histamine (an H_3 agonist) was observed on increasing the frequency of stimulation. The pA_2 value of the H_3 antagonist impromidine was, however, not altered at different stimulation frequencies; for a partial agonist only the maximal effect was influenced. These results indicate the existence of a receptor reserve at the H_3 autoreceptor.

During the last decade conclusive evidence has been gained for a definite function of histamine as a neurotransmitter in the central nervous system (cf. Schwartz et al 1982 for review). More recently it has been shown that, in rat brain, the release of histamine after tissue depolarization is under negative feedback control by a presynaptic autoreceptor (Arrang et al 1983). This so-called H_3-receptor has pharmacological characteristics different from H_1 and H_2 receptors. Histamine release and its blockade by exogenous histamine has been shown to be dependent on the conditions of stimulation, both after depolarization with K^+ and electrical stimulation (Arrang et al 1983; Van der Werf et al 1987a, b). In the present study this feature has been elaborated in more detail with the use of electrical stimulation applied to superfused brain slices of rats. Moreover, attention has been paid to the effects of impromidine (which antagonizes the response evoked by histamine) on the release of histamine under different conditions of stimulation. From the observations the existence of a frequency-dependent receptor reserve is postulated.

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Materials and methods

Chemicals and drugs. Histamine (HCl salt) was purchased from Aldrich (Belgium). Impromidine (HCl salt) was a gift from SK & F (UK). VUF8621 (a member of a series of side-chain derivatives of histamine: details to be published) was obtained from our laboratory stock. Dowex 50 WX4 (200–400 mesh) was obtained from Serva (Switzerland), L-[2,5-^{3}H]histidine (spec. act. ± 60 Ci mmol^{-1}) from Amersham International (UK). All other chemicals were from Merck (FRG) or Baker (The Netherlands).

Tissue preparation, superfusion and analysis of [^{3}H]histamine. The methods have been described by Van der Werf et al (1987a). Briefly the experimental setup was as follows. Male albino Wistar rats (190–200 g, TNO, The Netherlands) were decapitated, brains were removed and slices were prepared from the cerebral cortex. The slices were incubated in the presence of 150 μCi [^{3}H]histidine, washed to remove excess of [^{3}H]histidine and distributed among the 32 cells of a superfusion apparatus. Following equilibration, superfusion fractions were collected on chromatography columns. Depolarization of the slices was by electrical stimulation (biphasic 2 ms block pulses of 20 mA). In contrast to previous work, slices were depolarized only once. After superfusion, the slices were extracted by homogenization and centrifugation (Van der Werf et al 1987a).