Two novel prolactin release-inhibiting 8α-amino-ergolines

E. Flückiger, U. Briner, H.R. Bürki, P. Marbach, H.R. Wagner and W. Doepfner

Preclinical Research, Pharmaceutical Division, Sandoz Ltd, CH-4002 Basel (Switzerland), 7 September 1979

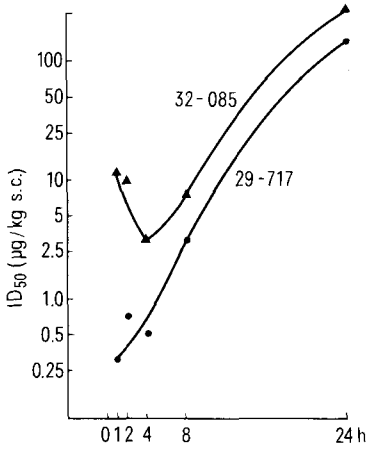
Summary. Prolactin secretion inhibition and changes in striatal dopamine metabolism in rats were compared after the administration of 8α-amino-ergoline CH 29-717 and 2 derivates. CQ 32-084 was similar to but less potent than CH 29-717, while 32-085, the l-methyl derivative, showed delayed dopaminomimetic effects.

In recent years a number of ergot compounds have been described which are dopaminomimetics. The compounds thus characterized belong either to the group of ergopeptines (e.g. bromocriptine[1-3]) or the clavines. Of the non-natural ergot group of 8α-amino-ergolines only 1 representative has been studied in detail, lisuride[3]. The prolactin secretion inhibitory property of another 8α-amino-ergoline, N,N-dimethyl-N′-(6-methyl-ergoline-8α-yl)-sulfamide hydrochloride (CH 29-717) has been reported[4,5]. We wish to extend the information about the dopaminomimetic property of this interesting family of ergot compounds by comparing the effects on prolactin secretion and dopamine metabolism of CH 29-717 and 2 new derivatives in rats. These are the compounds N,N-diethyl-N′-(6-methyl-ergoline-8α-yl)-sulfamide hydrochloride (=CQ 32-084) and N,N-dimethyl-N′-(1,6-dimethyl-ergoline-8α-yl)-sulfamide hydrochloride (=32-085). The 3 compounds are easily dissolved in water.

Methods. 3 of the 4 experimental methods used in this rat study (inhibition of basal prolactin (Prl) secretion, inhibition of ovum implantation, lactation inhibition) have recently been described in this journal[4]. DOPAC (3,4-dihydroxy-phenylacetic acid), a metabolite of dopamine, was measured in the striatum by a fluorimetric method[1,6].

Results. Results obtained for the 3 compounds in the 4 test systems are presented in table 1. It should be noted that in tests A and B the end-point of the experiment was 4 h after drug administration, while test C implies a duration of prolactin suppression of about 24 h[7], as does test D[8]. It can be seen that CQ 32-084 and 32-085 are less potent than the parent compound CH 29-717. Whereas the potency of CQ 32-084, as compared to CH 29-717, is reduced similarly in all 4 tests (3-5 times), the potency of 32-085 is reduced 8-10 times in the short-term experiments A and B, while in the other 2 test systems the reduction in potency is by a factor of 1.4 and 2.7 only. This discrepancy is unexpected. To explain it one could speculate that 32-085 is not fully active at first and becomes so only after metabolic conversion. Corroboration for the idea that the activity of 32-085 changes with time was sought from 2 sets of experiments where the duration of drug action was varied. The figure depicts the variation with time of ID_{50} values for serum prolactin suppression by CH 29-717 and 32-085. It can be seen that the potency of the former is maximal at 1 h after drug administration while the latter is most potent only at 4 h. The difference of potency between the 2 compounds becomes smaller with time. A similar time study, but using only 1 dose per compound, was done on the changes of striatal DOPAC concentration, the results of which appear in table 2. It shows that CH 29-717 and CQ 32-084 decrease DOPAC from the 1st to 8th h while the action of 32-085 is clearly biphasic. At 1 h after drug adiminstration DOPAC is nearly doubled, then wanes and only sinks below the level in the controls at 4 and 8 h.

Compound 32-085 differs from the other 2 compounds in an important way. Its action on dopamine metabolism in the striatum was biphasic with an unexpected increase of DOPAC after 1 and 2 h, indicating accelerated dopamine turnover, while at the same time prolactin secretion was inhibited, albeit not maximally. After 4 h DOPAC reduction and prolactin secretion-inhibition indicate that 32-085 acted now both at the pituitary and the striatal level, as expected of a dopaminomimetic agent. The differential change in time of the activities of 32-085 is unique and unexplained. Recently it was shown that a 1-methylated



Time dependence of prolactin secretion inhibitory potency of CH 29-717 and 32-085. Abscissa: time after drug administration. Ordinate: ID_{50} (µg/kg s.c.). The difference of potency between the compounds was as follows. 1 h: 40 times; 2 h: 14; 4 h: 6.4; 8 h: 2.4; 24 h: 1.9 times.

Table 1. Potencies of the 3 8α-amino-ergolines in 4 test systems

	A Inhibition of basal Prl secretion ID_{50} (4 h) (µg/kg s.c.)	B Reduction of DOPAC ED_{min} (4 h) (µg/kg s.c.)	C Inhibition of implantation ED_{50} (µg/kg s.c.)	D Inhibition of lactation ID_{50} (µg/kg oral)
CH 29-717	0.4	100	12.5	26
CQ 32-084	1.4	300	28	130
32-085	3.2	1000	18	70

Table 2. Time dependent changes of striatal DOPAC after CH 29-717, CQ 32-084 or 32-085

	Controls	DOPAC in % of controls (M±SD) Treated rats killed after 1	2	4	8 h
CH 29-717	100±9	72±4[b]	67±5[b]	58±7[b]	57±4[b]
(10 mg/kg s.c.)	(11)	(6)	(6)	(7)	(6)
CQ 32-084	100±6	84±11[a]	88±3[a]	76±5[b]	71±5[b]
(3.2 mg/kg s.c.)	(11)	(4)	(5)	(6)	(4)
32-085	100±5	193±21[b]	115±8[a]	84±5[b]	72±13[b]
(3.2 mg/kg s.c.)	(7)	(4)	(4)	(5)	(5)

Number of analyzed brains in parentheses. Significance by Student's t-test: a = $p < 0.01$, b = $p < 0.001$.

lysergic acid amide derivate, methysergide, suppressed prolactin secretion in the rat, probably only after its metabolic conversion[9], but central dopamine metabolism was not studied. It is difficult to assume that such a metabolic conversion (demethylation) is responsible for the differential effects observed with 32-085.

1 J.M. Vigouret, H.R. Bürki, A.L. Jaton, P.E. Züger and D.M. Loew, Pharmacology *16*, Suppl. 1, 156 (1978).
2 D.M. Loew, J.M. Vigouret and A.L. Jaton, in: Dopaminergic Ergot Derivatives and Motor Function, p. 129 Ed. K. Fuxe and D.B. Calne. Pergamon Press, Oxford 1979.
3 W. Kehr, R. Neumeister and R. Zimmermann, in: Dopaminergic Ergot Derivatives and Motor Function, p. 173. Ed. K. Fuxe and D.B. Calne. Pergamon Press, Oxford 1979.
4 E. Flückiger, U. Briner, W. Doepfner, E. Kovacs, P. Marbach and H.R. Wagner, Experientia *34*, 1330 (1978).
5 P. De Camilli, D. Macconi and A. Spada, Nature *278*, 252 (1979).
6 P.F. Spano and N.H. Neff, Analyt. Biochem. *42*, 113 (1971).
7 W. Wuttke and K.D. Döhler, in: Human Prolactin, p. 156. Ed. J.L. Pasteels and C. Robyn. Excerpta Medica, Amsterdam 1973.
8 E. Flückiger, in: Physiology of Mammary Glands, p. 71. Ed. A. Yokoyama, H. Mizuno and H. Nagasawa. Japan Scientific Press, Tokyo 1978.
9 S.W.J. Lamberts and R.M. MacLeod, Endocrinology *103*, 287 (1979).

Effect of orchidectomy and estradiol on acetylcholinesterase activity in rat brain areas and adenohypophysis

C.A. Iramain[1], G.N. Egbunike and J.O. Owasoyo

Departments of Biochemistry, Animal Science and Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan (Nigeria), 5 March 1979

Summary. Orchidectomy plus estradiol administration decreased acetylcholinesterase activity in the rat cerebral cortex and mesencephalon, while in the amygdala it was increased. In the adenohypophysis, orchidectomy increased enzyme activity, but subsequent estradiol treatment decreased it. The hypothalamus did not respond to either manipulation.

Cholinergic systems, which are known to play a significant role in normal autonomic neural function, can be modulated by many factors including hormones. Acetylcholine (ACh), the cholinergic neurotransmitter, has been implicated in the feedback regulation of gonadotropins[2]. Also, gonadal hormones play an important role in behavioural changes which are, in part, mediated through the cholinergic system of the CNS[3]. It is also known that acetylcholinesterase (AChE; EC 3.1.1.7), the hydrolytic enzyme for ACh, is of vital importance in the cholinergic neurohumoral transmission, while Moudgil and Kanungo[4] have shown that estradiol (E_2) increases AChE activity in the cerebral hemisphere and cerebellum of immature and adult female rats, at 4 h post-injection. In order to expand these observations, and also to check the influence of sex, we studied the effect of orchidectomy and E_2 on AChE activity in 4 brain areas, and the adenohypophysis in the male.

Material and methods. Adult male Wistar rats weighing 200–300 g were used. The animals were kept at 20–24 °C under a 12 h light period, starting from 07.00 h followed by a 12-h dark period, and fed standard pellet diet and water ad libitum. They were randomly divided into 3 groups of 8–10 animals; group 1: intact, group 2: orchidectomized (orchidex.) under ether anesthesia and used one or more weeks after, and group 3: orchidex. and given a single i.p. injection of 10 µg of E_2 (17 beta-estradiol; Sigma) per 100 g b.wt in 1 ml of 0.9% saline and used 4 h after the injection. The rats were killed by decapitation at a fixed time of the day (12.00 h) and the brains were quickly removed, freed of meninges and superficial blood vessels, and dissected on ice according to Gispen et al.[5] into: parietal cerebral cortex, mesencephalon, amygdala with overlying cortex pyriformis and hypothalamus. After removal of the brain from the skull case the neurohypophysis was separated in situ, and the adenohypophysis was removed from the sella turcica. A 2% homogenate (w/v) was prepared in 0.1 M ice-cold phosphate buffer (PB), pH 7.4, and AChE was assayed immediately by the spectrophotometric method of Ellman et al.[6] with minor modifications. In essence, the reaction mixture, in quartz cuvettes, contained 2.6 ml of PB, 0.4 ml of homogenate, 0.1 ml of dithiobisnitrobenzoate (0.01 M; Sigma), and 0.02 ml of acetylthiocholine iodide (0.075 M; Sigma) as substrate. The reaction was started by adding the substrate and the increase in absorbance was measured at 405 nm. The blank contained all the reactants except the substrate, and the AChE activity was calculated as µmole of substrate hydrolized/min/g of tissue. The results obtained were subjected to Student's t-test and are summarized in the table.

Results and discussion. It was found that orchidectomy plus E_2 significantly decreased AChE activity in the cerebral cortex and mesencephalon, while in the amygdala the activity was increased. In the adenohypophysis, orchidectomy drastically increased AChE activity, but subsequent E_2

AChE activity in male rat brain areas and adenohypophysis (average rates of hydrolysis of acetylthiocholine)

Area	Rates (µmole/min/g of tissue ± SEM)		
	Group 1 (n = 8) intact	Group 2 (n = 10) orchidex.	Group 3 (n = 8) orchidex. + E_2
Cerebral cortex	5.76 ± 0.15	5.73 ± 0.98	4.83 ± 0.39*
Mesencephalon	13.01 ± 0.71	10.83 ± 1.52	9.78 ± 1.50*
Amygdala	8.06 ± 1.24	6.88 ± 0.78***	10.44 ± 0.78*
Hypothalamus	7.68 ± 0.15	6.92 ± 1.54	6.58 ± 0.95
Adenohypophysis	1.03 ± 0.25**	3.93 ± 0.96***	1.35 ± 0.19

* Difference from group 1: significant ($p < 0.05$); ** Difference from group 2: significant ($p < 0.05$); *** Difference from group 3: significant ($p < 0.05$).