REFERENCES

1. Laskowski M and Kato I, Protein inhibitors of proteinases. *Annu Rev Biochem* **49**: 593–626, 1980.
2. Marks N, Berg MJ and Benuck M, Preferential action of rat brain cathepsin B as a peptidyl dipeptidase converting pro-opiod oligopeptidases. *Arch Biochem Biophys* **249**: 489–499, 1986.
3. Rockett KA, Playfair JHL, Ashall F, Targetl GAT, Anjliker H and Shaw E, Inhibition of intraerythrocytic development of *P. falciparum* by protease inhibitors. *FEBS Lett* **295**: 257–260, 1990.
4. Vonderfecht SL, Miskuff RL, Wee SB, Sato S, Tidwell RR, Geratz JD and Yolken RH, Protease inhibitor suppress the *in vitro* and *in vivo* replication of retroviruses. *J Clin Invest* **82**: 2011–2016, 1988.
5. Schnebli HP and Burger MM, Selective inhibition of growth of transformed cells by protease inhibitors. *Proc Natl Acad Sci USA* **69**: 3825–3827, 1972.
6. Chou IN, Black PH and Roblin RO, Non-selective inhibition of transformed cell growth by a protease inhibitor. *Proc Natl Acad Sci USA* **71**: 1748–1752, 1974.
7. Troll W, Frankel K and Wiesner R, Protease inhibitors as anticarcinogens. *J Natl Cancer Inst* **73**: 1245–1250, 1984.
8. Troll W, Wiesner R and Frenkel K, Anticarcinogenic action of protease inhibitors. *Adv Cancer Res* **49**: 265–283, 1987.
9. Troll W, Protease inhibitors interfere with the necessary factors of carcinogenesis. *Environ Health Perspect* **81**: 59–62, 1989.
10. Weed HG, McGandy, RB and Kennedy AR, Protection against dimethylhydrazine induced adenomatous tumours of the mouse colon by the dietary addition of an extract of soybeans containing the Bowman–Burk protease inhibitor. *Carcinogenesis* **6**: 1239–1241, 1985.
11. Mokhtar NM, El-Aaser AA, El-Bolkainy MN, Ibrahim AA, El-Din NB and Moharram NZ, Effect of soybean feeding on experimental carcinogenesis III. Carcinogenicity of nitrate and dibutylamine in mice. A histological study. *Eur J Cancer Clin Oncol* **24**: 403–411, 1988.
12. Schelp FB and Pongpaew P, Protection against cancer through nutritionally induced increase of endogenous proteinase inhibitor—a hypothesis. *Int J Epidemiol* **17**: 287–292, 1988.
13. Busby WF and Wogan GN, Aflatoxins. In: *Chemical Carcinogens* (Ed. Searle CE), pp. 945–1136. American Chemical Society, Washington, DC, 1984.
14. Sohonie K and Ambe KS, Crystalline trypsin inhibitor from the Indian field bean and the double bean. *Nature* **175**: 508–509, 1955.
15. Kunitz M, Crystalline soybean trypsin inhibitor II. General properties. *J Gen Physiol* **30**: 291–310, 1947.
16. Quillardet P and Hofnung M, The SOS chromotest, a colorimetric bacterial assay for genotoxins: procedures. *Mutat Res* **147**: 65–78, 1985.
17. Ames BN, McCann J and Yamasaki E, Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutat Res* **31**: 347–364, 1975.
18. Miller JH, *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1972.
19. Fritz H, Trantschold I and Werle E, Protease inhibitors. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 1064–1079. Academic Press, New York, 1974.
20. Huisman O and d'Ari R, an inducible DNA replication cell division coupling mechanism in *E. coli*. *Nature* **290**: 797–799, 1981.
21. Sloane BF and Honn KY, Cysteine proteinases and metastases. *Cancer Metastasis Rev* **3**: 249–263, 1984.

Biochemical Pharmacology, Vol. 43, No. 8, pp. 1882–1884, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
Pergamon Press Ltd

Stimulation of dopamine D_1 receptors by the D_2 agonist CV 205-502 in bovine retina homogenates

(*Received* 27 *September* 1991; *accepted* 16 *January* 1992)

CV 205-502, an octahydrobenzo(*g*)quinoline, combines the essential dopaminomimetic pharmacophore of apomorphine with the long duration of action and the good oral activity of the ergolines [1]. So far it has been used on the one hand as a high-affinity selective dopamine (DA*) D_2 receptor agonist ligand in autoradiographic studies [2, 3], and on the other hand as a prolactin inhibitor and an alternative to bromocriptine in the treatment of pituitary prolactinomas and of hyperprolactinaemia [4, 5]. In the autoradiographic studies, [^{3}H]CV 205-502 binding was displaced with high affinity by DA D_2 receptor agonists or antagonists such as apomorphine, spiroperidol, *S*(−)-sulpiride and (+)-butaclamol, but not by the inactive enantiomer (−)-butaclamol; the DA D_1 receptor agonist SKF 38393 and the D_1 antagonist *R*(+)-SCH 23390 were also ineffective [3]. However, binding studies in the rat striatum have shown that [^{3}H]CV 205-502 is very weakly displaced by SCH 23390, with an IC_{50} of 1.98 ± 0.7 μM [2]. To our knowledge, no direct biochemical evidence has so far been provided to show that CV 205-502 is able to stimulate DA D_1 receptors positively linked to adenylate cyclase [6] and to concomitantly generate dose-dependent increases in cAMP concentrations.

The retina embodies many of the features that are distinctive of the central nervous system in general and does so within a structure that is relatively simple. Its easy isolation and remarkable survival as well as the identification of DA as a retinal neurotransmitter make it a useful model for the biochemical characterization of the DA-sensitive adenylate cyclase (Refs 7; 8 for a review and references therein). Bovine retina homogenates were thus used in this investigation to test whether CV 205-502 could possibly induce the formation of cAMP through the stimulation of DA D_1 receptors.

* Abbreviations: DA, dopamine; EtOH, ethanol.

Materials and Methods

To determine the activity of the DA-sensitive adenylate cyclase in homogenates of bovine retina, we have used the procedure of Markstein *et al.* [9] with the following modifications: (1) 100 μL of retina homogenates were added to 350 μL of the buffer (48.8 mM Tris–HCl, 2 mM $MgCl_2$, 0.45 mM ethyleneglycolbis(aminoethylether)tetra-acetate, 0.1 mM isobutylmethylxanthine, pH 7.4) containing a DA D_1 receptor agonist and/or antagonist, except in the controls. (2) The mixture was kept on ice for 13.5 min before a rapid conditioning at 30° for 1 min. The final 3-min incubation was then started with 50 μL of a solution containing 5 mM ATP and 0.1 mM guanosine 5′-(β,γ-imido)triphosphate. (3) After termination of the enzymatic reaction, the homogenates were centrifuged at 4° for 30 min (1200 *g*). The amounts of protein in the pellets and of cAMP in the supernatants were measured according to the methods of Lowry *et al.* [10] and of Brown *et al.* [11], respectively. Ethanol (EtOH) was needed to solubilize the CV 205-502. A 2.315 mM stock solution was made in the above-mentioned buffer containing 30% (v/v) of EtOH.

Results

Figure 1 shows that CV 205-502 stimulated adenylate cyclase activity in a dose-dependent way. The results are given in per cent increases in cAMP induced by CV 205-502 over controls (100%). Concentrations of CV 205-502 under 3×10^{-6} M were totally ineffective ($P > 0.05$). The effects on cAMP accumulation were maximal at concentrations of CV 205-502 superior to 10^{-5} M. At 3×10^{-5} M and 10^{-4} M concentrations of CV 205-502, the cAMP increases were not statistically different (133.2% ± 2.6 and 123.9% ± 4.7 over controls). For example, DA used at 10^{-4} M concentrations led to an extra 119.47% ± 14.7 increase in cAMP over controls in comparison to 3×10^{-5} M CV 205-502 (see also Ref. 12).

In addition, 10–100 μM quinpirole, a classical D_2 receptor agonist, was unable to modify the cAMP levels (data not shown). Figure 2 shows the dose-dependent (10^{-9}–10^{-4} M) effects of a DA D_1 receptor antagonist (e.g. SCH 23390) on the activity of adenylate cyclase maximally stimulated by a fixed concentration of CV 205-502 (3×10^{-5} M). SCH 23390 was effectively able to lower the increase in cAMP levels induced by CV 205-502 in a dose-dependent manner. A maximal effect of SCH 23390 was already observed at 10^{-7} M concentrations. Interestingly, the enzymatic activity observed in the homogenates containing 3×10^{-5} M CV 205-502 and concentrations of SCH above 10^{-6} M was significantly lower than in the controls ($P < 0.02$).

The effects of a fixed concentration of SCH 23390 (10^{-5} M) were also studied in the presence of various concentrations of CV 205-502 (data not shown). Under such conditions, a decrease in the enzymatic activity was also observed in the homogenates containing CV 205-502 and SCH 23390 compared to the homogenates containing only CV 205-502. Again, in the presence of SCH 23390, the enzymatic activity was lower than in the controls ($P < 0.025$).

Discussion

CV 205-502, a non-ergot or -ergoline derivative, has been shown to be a DA D_2 receptor agonist [1] and a weak DA D_1 receptor agonist in binding studies [2]. In homogenates of bovine retina, it is now possible to investigate the effect of CV 205-502 on the enzyme adenylate cyclase. CV 205-502 was able to increase the cAMP levels in a concentration-dependent manner, with a maximal effect at 3×10^{-5} M, as expressed by an increase of 133.24 ± 2.6% over controls, and an ED_{50} of about 10^{-5} M. Thus, CV 205-502 is less potent and much less efficacious than the full agonist DA tested under similar conditions. The cAMP increase induced by 3×10^{-5} M CV 205-502 was partially inhibited by concentrations of SCH

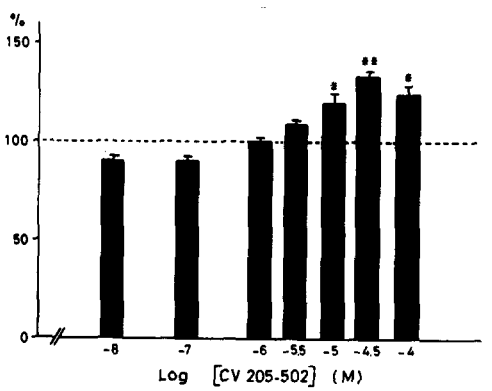

Fig. 1. CV 205-502-stimulated adenylate cyclase activity in bovine retina homogenates. EtOH was present at concentrations of 0.04, 0.13, 0.4 and 1.3% (v/v) in assay tubes containing 3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M CV 205-502, respectively, as well as in the corresponding controls (see Materials and Methods for other technical details). The dose-dependent effects of CV 205-502 are expressed as % increases over controls (dashed line, 100%) ± SEM (N = 5). Statistical analysis was assessed by the Student's *t*-test (*$P < 0.005$; **$P < 0.001$ vs controls).

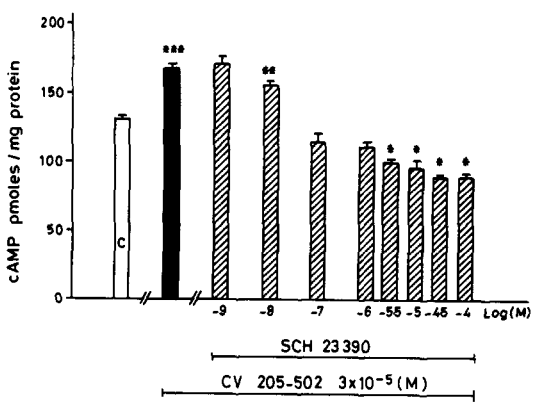

Fig. 2. Dose-dependent inhibition by SCH 23390 of the adenylate cyclase activity stimulated by 3×10^{-5} M CV 205-502. EtOH was present at 0.4% (v/v) in all assay tubes including the controls (C). Results are expressed as pmoles cAMP per mg protein (means ± SEM, N = 5, see Materials and Methods for other technical details). The statistical analysis was assessed by the Student's *t*-test (*$P < 0.02$ vs controls; **$P > 0.05$ vs CV 205-502 alone; ***$P < 0.001$ vs controls).

23390 higher than 10^{-8} M, while its total suppression or an enzymatic activity inferior to that of controls has been observed when SCH 23390 was used at supramaximal concentrations ($\geq 3 \times 10^{-6}$ M).

Thus our present report unequivocally demonstrates that CV 205-502, a potent DA D_2 agonist in various systems of investigation *in vivo* [4, 5, 13] and *in vitro* [2, 3], is also a partial agonist at D_1 receptors *in vitro*. Consequently, this new chemical type of DA agonist should in fact be considered as a non-selective DA receptor agonist, implying a broader pharmacological spectrum of activity and/or possible additional side effects than were claimed initially, when it was administered to humans [13].

Furthermore, when DA receptors were blocked totally by high concentrations of SCH 23390 ($\geq 3 \times 10^{-6}$ M), the levels of cAMP in response to CV 205-502 appeared to be significantly lower than those observed in the controls (Fig. 2). The simplest and most attractive explanation is that under these experimental conditions (i.e. total blockade of D_1 receptors positively linked to adenylate cyclase activity [14]), the stimulation by CV 205-502 of DA D_2 receptors, which are negatively coupled to adenylate cyclase with a concomitant decrease in cAMP concentrations [6], can be observed. In fact, although not significantly different from the controls, small decreases in cAMP levels were also apparent in the presence of 10^{-7} to 10^{-6} M concentrations of SCH 23390. It would thus appear that when D_1 receptors were effectively and completely blocked with higher concentrations of SCH 23390, CV 205-502 was able to inhibit the adenylate cyclase activity. Contrasting with other unsuccessful attempts to reveal such DA D_2 receptors in retina homogenates compared with the intact cells of most species [8], as well as in rat cerebellar membranes [15], the unmasking of such receptors appears to be feasible in the presence of CV 205-502. It has been shown however that the negative biochemical signal can only be observed in the presence of an active Ca^{2+}–calmodulin complex and in the absence of guanosine 5′-(β,γ-imido)triphosphate [15]. Since these requirements were not met under our experimental conditions, further investigations related to the simultaneous biochemical and pharmacological detection of both DA D_1 and D_2 receptors should be undertaken in bovine retina homogenates.

In summary, CV 205-502, a known potent DA D_2 receptor agonist and presumably a weak DA D_1 receptor agonist, was shown to stimulate effectively DA-sensitive adenylate cyclase (e.g. D_1 receptors) in homogenates of bovine retina in a dose-dependent manner. In addition, the stimulating effect of CV 205-502 could be completely blocked by a selective DA D_1 receptor agonist such as SCH 23390 used at 10^{-7} M. This study provides the first direct biochemical evidence *in vitro* that CV 205-502 is also a partial DA D_1 receptor agonist, and thus should definitely be considered as a non-selective DA receptor agonist. Finally, CV 205-502 may represent a new pharmacological agent to detect simultaneously DA receptors that are positively (D_1) or negatively (D_2) coupled to adenylate cyclase activity.

Acknowledgements—This work was supported by the Swiss National Science Foundation (SNSF) Grant 31-25625-88 to M. S. CV 205-502 was kindly provided by Sandoz Ltd. The authors wish to thank Ms Gisèle Gilliéron for skillful technical assistance, as well as Mr Fred Pillonel for excellent graphical work. We are also very grateful to Olivier Bugnon and Senyo Ofori for their continuous enthusiasm and critical help.

* Corresponding author. Tel. (41) 22-22-92-79; FAX (41) 22-47-33-34.

Department of Pharmacology
University Medical Center
1211 Geneva 4
and †School of Pharmacy
University of Lausanne
1015 Dorigny
Switzerland

SASKIA DE RAAD*
MICHEL SCHORDERET†

REFERENCES

1. Nordmann R and Petcher TJ, Octahydrobenzo(*g*)-quinolines: potent dopamine agonists which show the relationship between ergolines and apomorphine. *J Med Chem* **28**: 367–395, 1985.
2. Charuchinda C, Supavilai P, Karobath M and Palacios JM, Dopamine D_2 receptors in the rat brain: autoradiographic visualization using a high-affinity selective agonist ligand. *J Neurosci* **7**: 1352–1360, 1987.
3. Camps M, Cortés R, Gueye B, Probst A and Palacios JM, Dopamine receptors in human brain: autoradiographic distribution of D_2 sites. *Neuroscience* **28**: 275–290, 1988.
4. Homburg R, West C, Brownell J and Jacobs HS, A double-blind study comparing a new non-ergot, long-acting dopamine agonist, CV 205-502, with bromocriptine in women with hyperprolactinaemia. *Clin Endocrinol* **32**: 565–571, 1990.
5. Barnett PS, Dawson JM, Butler J, Coskeran PB, Maccabe JJ and McGregor AM, CV 205-502, a new non-ergot dopamine agonist, reduces prolactinoma size in man. *Clin Endocrinol* **33**: 307–316, 1990.
6. Clark D and White FJ, Review: D1 dopamine receptor—The search for a function: a critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. *Synapse* **1**: 347–388, 1987.
7. Brown JH and Makman MH, Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine-3′:5′-cyclic monophosphate formation in intact retina. *Proc Natl Acad Sci USA* **69**: 539–543, 1972.
8. Schorderet M and Nowak JZ, Retinal dopamine D_1 and D_2 receptors: characterization by binding or pharmacological studies and physiological functions. *Cell Mol Neurobiol* **10**: 303–325, 1990.
9. Markstein R, Enz A, Vigouret JM, Jaton A, Closse A, Briner U and Gull P, Biochemical, behavioural and endocrine effects of CK 204-933, a novel 8β-ergolene. *J Neural Transm* **69**: 179–199, 1987.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Brown BL, Albano JDM, Ekins RP and Sgherzi AM, A simple and sensitive saturation assay method for the measurement of adenosine 3′,5′-cyclic monophosphate. *Biochem J* **121**: 561–562, 1971.
12. Schorderet M, de Raad S and Ofori S, Biochemical and pharmacological modulation of the dopamine sensitive adenylate cyclase in homogenates of bovine retina. *Soc Neurosci Abstr* **17**: 1354, 1991.
13. Gaillard RC, Abeywickrama K, Brownell J and Muller AF, Specific effects of CV 205-502, a potent non ergot dopamine agonist, during a combined anterior pituitary function test. *J Clin Endocrinol Metab* **68**: 329–335, 1990.
14. Schorderet M, Receptors coupled to adenylate cyclase in isolated rabbit retinas. *Neurochem Int* **14**: 387–395, 1989.
15. Cooper DMF, Ahlijanian MK and Perez-Reyes E, Calmodulin plays a dominant role in determining neurotransmitter regulation of neuronal adenylate cyclase. *J Cell Biochem* **36**: 417–427, 1988.