INHIBITION OF CYTOCHROME P450c11 BY BIOGENIC AMINES AND AN AZIRIDINE PRECURSOR, 2-(4-ACETOXYPHENYL)-2-CHLORO-N-METHYL-ETHYLAMMONIUM CHLORIDE

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ABSTRACT

The interaction of several biogenic amines and Compound A (2-(4-acetoxyphenyl)-2-chloro- N-methyl-ethylammonium chloride), an analogue of the active substance in a HPLC fraction isolated from the shrub, *Salsola tuberculatiformis* Botsch., with cytochrome P450c11 was investigated. Noradrenaline, octopamine and Compound A inhibited the type I DOC induced difference spectrum of P450c11 and elicited a type II difference spectrum when added alone. The Ks-values for noradrenaline, octopamine, and Compound A were 0.8 mM, 0.16 mM and 0.36 mM, respectively. Dopamine, adrenaline and synephrine did not interact with, or inhibit, P450c11. Further investigation of Compound A indicated that it is a mixed inhibitor of sheep P450c11 with a stronger competitive (Kic = 106-110 μM) than uncompetitive (Kiu = 667-737 μM) element.

INTRODUCTION

During an investigation of bioactive substances in the Namibian shrub, *Salsola tuberculatiformis* Botsch., high performance liquid chromatography (HPLC) analyses of an 11β-hydroxylase (P450c11) inhibiting fraction yielded two potentially active fractions. Further analyses of these two fractions indicated the presence of an aziridine in the first, and dopamine in the second fraction [1]. The aziridine containing fraction, labeled S2, inhibited the binding of deoxycorticosterone to P450c11 [2]. The active substance(s) in this fraction were, however, extremely unstable and activity was lost during exposure to light, oxygen and high pH. The major breakdown product of S2 was synephrine, which did not inhibit P450c11. This suggested that S2 contained a P450c11 inhibiting aziridine, aziridine precursor or biogenic amine. The presence of dopamine in the second fraction was therefore interesting and although dopamine did not inhibit P450c11, the possibility that it was a breakdown product of a more active biogenic amine precursor in the plant could not be ruled out. Aziridines are found in nature as mitomycins and are highly reactive N-tricyclic compounds used in cancer treatment [3]. The reactivity of aziridines prompted the use of more stable precursors in clinical studies [4]. The presence of the breakdown product, synephrine, and data obtained from masspectrometry (MS) and nuclear magnetic resonance (NMR) studies prompted the syntheses of a more stable precursor, 2-(4-acetoxyphenyl) 2-chloro-N-methyl- ethylammonium chloride (Compound A), to assist in the investigation of the unstable active substance in S2 [5]. The influence of Compound A and a number of structurally related biogenic amines on ovine P450c11 was investigated. Spectral binding constants were determined for octopamine, noradrenaline, and Compound A. The mechanism of interaction of Compound A with P450c11 was further investigated with the aid of substrate induced difference spectra.

MATERIALS AND METHODS

Substrate Induced Difference Spectra

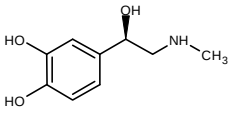
Lyophilised mitochondrial powder from sheep adrenals was used as a source of cytochrome P450c11 and the spectral assays were performed as previously published [2,5]. Compound A was prepared in PBS, immediately added to the test system, and spectra were recorded within 5 minutes; the biogenic amines were prepared in water. Two types of spectral assays were done: (1) substrate induced difference spectra by octopamine, noradrenaline, and Compound A and (2) inhibition of DOC-induced difference spectra by the same compounds. To determine the type of inhibition by Compound A spectral data was analyzed by nonlinear regression to determine sKmapp and sVmaxapp. The uncompetitive element (sKiu) was calculated by plotting 1/sVmaxapp vs inhibitor concentration (I) and the competitive element (sKic) calculated using the following formula [6]:

$$sKmapp = sKm (1+I/sKic) / 1+I/sKiu$$

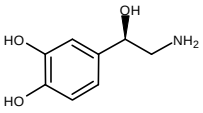
RESULTS AND DISCUSSION

Figure 1A shows the interaction of noradrenaline, octopamine, and Compound A with P450c11[1]. All three compounds inhibited the binding of DOC and elicited a Type II difference spectrum when added to the preparation in the absence of DOC. The Ks (spectral association constant) for the two biogenic amines and Compound A were determined using the double reciprocal plots shown in Figure 1B. The Ks-values for noradrenaline, octopamine, and Compound A were 0.8mM, 0.16mM and 0.36 mM, respectively. Structurally related amines, dopamine, adrenaline and synephrine, showed no inhibition of, or interaction with ovine adrenal P450c11 (results not shown). The results obtained with the biogenic amines were useful in designing the model compound, Compound A, as it indicated the importance of a functional group at position 2, adjacent to the

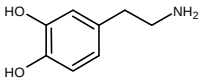
phenyl ring. The catechol arrangement on the ring is not obligatory for activity as the monohydroxy octopamine was more active than the catechol, noradrenaline. These results also implied that a demethylated nitrogen was important for P450c11 interaction. However, the studies with Compound A indicated that this was not always the case and that activity may be modulated depending on the nature of the functional group at position 2.



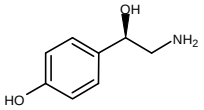
Adrenaline



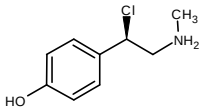
Nor-adrenaline



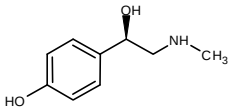
Dopamine



Octopamine



Compound A



Synephrine

Investigation of the substrate concentration dependent spectral changes in the DOC-induced difference spectrum, indicated that Compound A is a mixed inhibitor of sheep P450c11 with a stronger competitive (Kic = 106-110 μM) than uncompetitive (Kiu = 667-737 μM) element (Figure 2).

REFERENCES:

1. Van der Merwe KJ, de Kock SS, Swart P, Fourie L. 1991 Biochem Soc Trans 19:432s.

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[1]The two biogenic amines had no significant interaction with P450scc and it can be assumed that all the interactions between the amines and P450 could be attributed to P450c11 (unpublished).

2. Swart P, van der Merwe KJ, Swart AC, Todres PC, Hofmeyr J-HS. 1993 Planta Med 59: (2) 139-143.
3. Dermer OC, Ham GE. 1969 Ethylenimine and other aziridines: Chemistry and Applications. Academic Press, New York, pp. 394-443.
4. Calabresi P, Chabner BA. 1990 Antineoplastic agents. In: Gilman AG, Rall TW, Nies ASD, Taylor P (eds) Goodman and Gilman's The pharmacological basis of therapeutics. Pergamnon Press, New York, pp. 1209-1263.
5. Louw A, Swart P, de Kock SS, van der Merwe KJ. 1996 Biochem Pharmacol 53: 189-197.
6. Cornish-Bowden A. 1995 Fundamentals of Enzyme Kinetics, Portland Press Ltd, London.

FIGURE 1

Interaction of noradrenaline, octopamine, and Compound A with ovine P450c11 (A) Substrate induced difference spectra and (B) double reciprocal plots to determine Ks.

FIGURE 2

Spectral changes in the DOC-induced difference spectrum by varying amounts (2-50 μM) of substrate (DOC) was measured in the presence of different concentrations (0-250 μM) of Compound A. Spectra were recorded 5 min. after adding Compound A prepared in PBS.

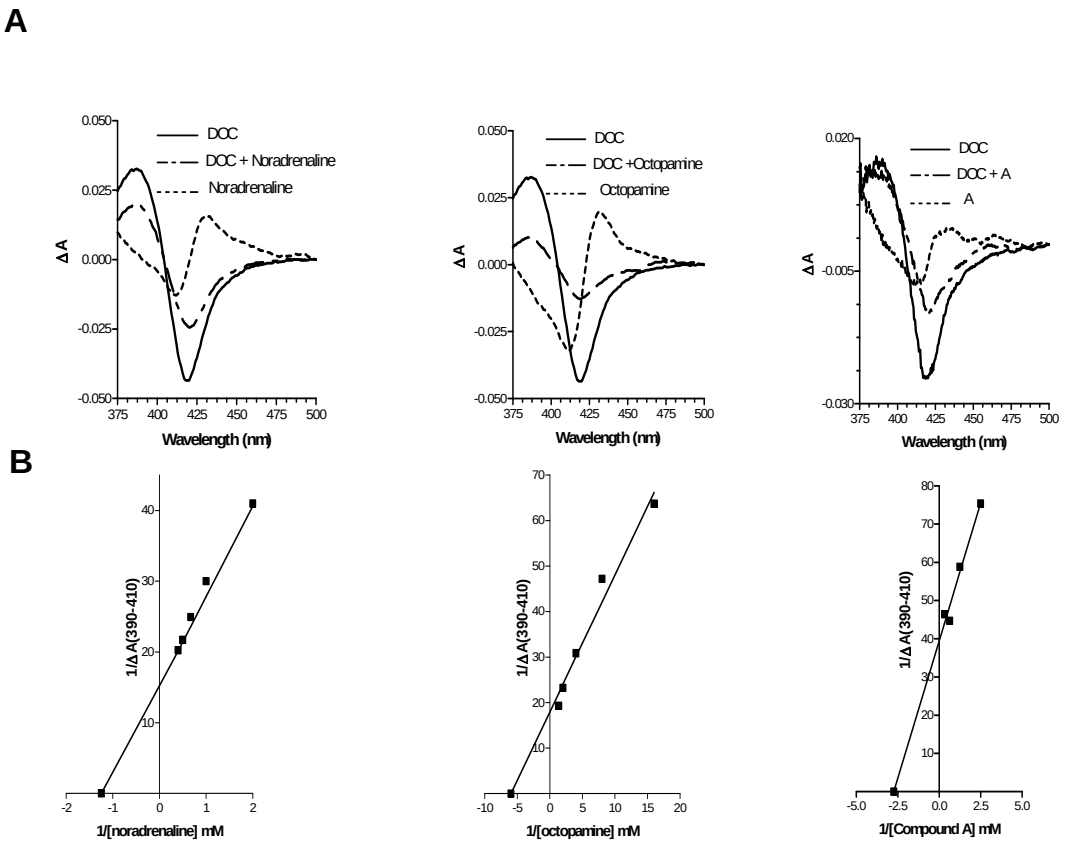
A
DOC
DOC + Noradrenaline
Noradrenaline
ΔA
Wavelength (nm)
DOC
DOC +Octopamine
Octopamine
ΔA
Wavelength (nm)
DOC
DOC + A
A
ΔA
Wavelength (nm)
B
1/Δ A(390-410)
1/[noradrenaline] mM
1/Δ A(390-410)
1/[octopamine] mM
1/Δ A(390-410)
1/[Compound A] mM

Fig 1

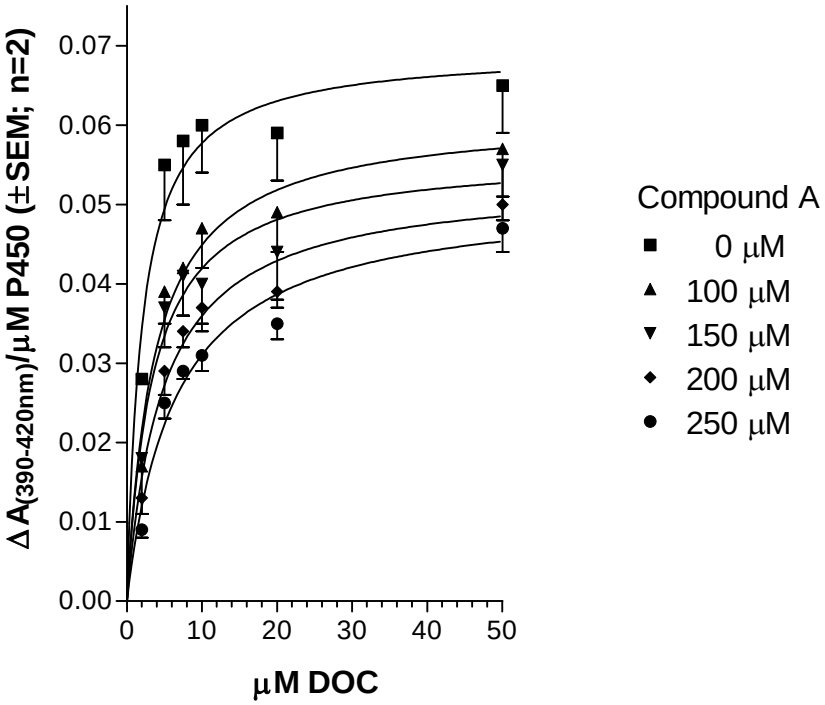
ΔA(390-420nm)/μM P450 (±SEM; n=2)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0
10
20
30
40
50
μM DOC
Compound A
0 μM
100 μM
150 μM
200 μM
250 μM

Fig 2