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ANTINOCICEPTIVE ACTIVITY OF CHROMOPHORIC CHAIN SUBSTITUTED HEMICYANINOCOLOURANTS

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Abstract: Some of the chromophoric chain substituted hemicyaninocolourants (CCHCs) were synthesized and confirmed on the basis of nitrogen analysis. These were tested for their antinociceptive activity in albino rats against tail flick technique and sodium chloride induced writhing test. Test compounds were given in graded doses (10, 20 and 50 mg/kg, intraperitoneally) and compared with morphine and aspirin as standard controls. Two compounds CCHC-1 and CCHC-2 showed antinociceptive activity in a dose-dependent manner in both the experimental models. The compound CCHC-3 did not exhibit antinociceptive activity to any significant extent.

Key words: hemicyaninocolourants (CCHCs) antionociception

INTRODUCTION

Dyes or colouring substances are known since antiquity. Human beings incorporated dyes into ceremonies marking births, marriages, deaths, wars and illnesses throughout the recorded history. People also used them traditionally for non-medical purposes to colour polymer substrates like textiles and plastics but their multidimensional uses have only been revealed in the present century. In therapeutics, dyes have been used mainly in the treatment of infectious diseases as intestinal and urinary antiseptics, antimalarial, antimicrobial and fungicidal agents (1).

Recently, cyaninocolourants have been tried in photodynamic therapy of malignant tumors (2, 3). Few azo dyes like pyridium and clofazimine have shown analgesic and antiinflammatory actions (4). This promoted us to evaluate the analgesic property of newly synthesized colour compounds, hemicyaninocolourants to know whether these compounds too have parallal activities to azo dyes. Two experimental models were used to screen antinociceptive activity of hemicyaninocolourants.

METHODS

Synthesis of chromophoric chain substituted hemicyaninocolourants (CCHCs): Three CCHCs were synthesized by the method of Hamer (5). The synthesis was carried out in 3 steps :-

(1) Preparation of quaternary bases (QBs) : First the QBs were prepared. Each QB was synthesized in two steps :-

Step I: Preparation of quinaldines: Three quinaldines were prepared from their respective basis i.e. p-chloroaniline, p-

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bromoaniline and p-anisidine and accordingly named as 6-chloro, bromo and methoxy quinaldines.

Step II: Combination of quinaldine with methyliodide: This combination gave quinaldinemethiodide. Thus QBs were produced and according to their substitution (Cl, Br and methoxy groups) were given the names QB-1, QB-2 and QB-3.

(2) Preparation of ketones: In its preparation, it included 2 phases:-

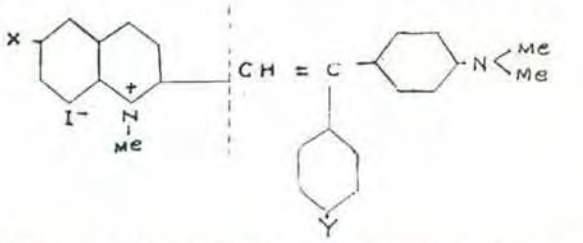
(i) Preparation of p-nitrobenzanilide: The method of Shah, Deshpande and Chaube modified by Jha et al (6) was used.

(ii) Preparation of 4-dimethylamino 4-nitrobenzophenone: This was prepared by combining p-nitrobenzanilide with dimethylaniline.

(3) Thermocatalytic condensation of bases and ketones: By refluxing the QBs with ketones, 3 compounds (CCHC-1, CCHC-2 and CCHC-3) were produced. Michler's ketone was used for condensation.

Chemistry of CCHCs: The chromophoric chain substituted hemicyaninocolourants carry a quaternary heteroaromatic moiety in one terminal and next aromatic system, having exocyclic tertiary nitrogen atom in other terminal of conjugated methine chain.

Animals: Wistar albino rats of either sex weighing between 175-225 g were used. The



(DIMETHYL AMINOPHENYL β-PHENYLIODOMETHYL) QUINOLINES

Fig. 1: Showing chemical structure of hemicyanino-colourants.

rats were kept under standard conditions of food and environment. The age of animals ranged from 75-90 days.

Drug preparation and treatment: Since CCHCs are insoluble in water, a number of solvents like ethanol, acetone, propylene glycol, polyethylene glycol-400 and 600, dimethyl formamide and tween-80 were considered to dissolve the compounds. A combination of polyethylene glycol-600 (25%) and ethanol (10%) were found as most suitable solvent. Therefore, to prepare the solution, CCHCs were dissolved in polyethylene glycol-600 (25%) and few drops of ethanol (10%) was added to get the clear solution of drugs. Each compound was given in the doses of 10 mg, 20 mg and 50 mg/kg by intraperitoneal route. The doses were selected by preliminary trial and having submaximal action. Morphine hydrochloride was dissolved in distilled water to get the solution of 1 mg/ml

TABLE I: Showing analytical data of CCHCs.

Compound No.	*-X-*	*-Y-*	*Molecular formula*	*mp °C*	*Yield (%)*	*Nitrogen (%)* *Found*	*Calculated*
1) CCHC-1	Cl	NO2	NO2C26H23C1IN302	204	44.5	7.31	7.35
						28.42	Cl+I:28.4
2) CCHC-2	Br	NO2	NO2C26H23BrIN302	210	46.5	6.72	N:6.82
						33.65	Br+I:33.6
3) CCHC-3	MeO	NO2	NO2C27H26MeOIN303	214	47.0	7.36	N:7.41
						22.34	I:22.4

concentration. It was administered in the dose of 10 mg/kg by intraperitoneal injection. Acetyl salicylic acid (aspirin) was prepared as 2% aqueous suspension in gum acacia and used in the dose of 50 mg/kg by oral route as standard drug only in writhing test.

For each drug, animals were divided into five groups of 10 each. Group I and II received distilled water and vehicle respectively and served as control whereas groups III, IV and V were administered the 3 graded doses of the test compounds.

Two methods were employed to evaluate the analgesic activity.

(1) Tail flick method: The Techno-hot wire analgesiometer was used to screen the analgesic activity of CCHCs compounds by the method of D'Amour and Smith (7). Before starting the experiment, rats were subjected to preliminary screening. Those showing variation of more than 1 second between 2 reaction times at 15 minutes interval or more than 3 seconds from group mean were discarded. The rats were kept in rat holder and middle section of tail was placed on the wire. After sometimes when animals adapted to new environment, the analgesiometer was switched on. The time when animal withdraws the tail (reaction time) was noted. The increase in reaction time in drug treated group indicates the antinociceptive activity.

(2) Sodium chloride induced writhing test: The method of Fukawa et al (8) was followed. The writhing was induced by intraperitoneal administration of 0.3 ml of 4% NaCl. The writhing consisted of a wave of constriction and elongation of abdominal musculature followed by extension of hind limbs.

TABLE II : Effect of CCHCs on reaction time in rats.
(by Tail flick method)

Group (n=10)	*Dose (ml+,mg/kg,ip)*	*Reaction time (seconds) Mean ± SEM*			
		Before	*After*		
			15 min	*30 min*	*45 min*
I Control					
D.W.	10+	7.41+0.11	7.42+0.16	7.48+0.20	7.43+0.17
Vehicle	10+	7.17+0.11	7.75+0.13	8.33+0.16	7.66+0.12
II Test drugs:					
CCHC-1	10	7.92+0.24	10.36+0.22	12.00+0.18	12.10+0.21
	20	7.83+0.21	12.92+0.24*	16.38+0.28**	17.66+0.15**
	50	7.79+0.15	29.83+0.11***	29.83+0.11***	30.00+0.00***
CCHC-2	10	7.33+0.22	8.41+0.26	9.33+0.20	7.37+0.28
	20	7.42+0.18	11.33+0.24	14.25+0.15**	10.22+0.24
	50	7.22+0.23	28.78+0.25***	29.96+0.19***	28.75+0.15***
CCHC-3	10	7.80+0.20	9.76+0.23	9.23+0.24	9.10+0.18
	20	7.46+0.22	9.88+0.25	9.39+0.17	9.17+0.22
	50	7.66+0.12	10.57+0.28	10.42+0.29	10.23+0.15
III Standard drug:					
Morphine	10	7.83+0.17	29.70+0.19***	29.78+0.19***	29.66+0.21***

D.W. = Distilled Water *P<0.05 **P<0.01 **P<0.001
n = Number of animals

The rats were selected by preliminary screening. The animals showing positive response within 10 seconds were included in the study. Abolition of writhing response was considered to be criteria of analgesic activity of colourants.

RESULTS

(1) By tail flick method: CCHCs were administered intraperitoneally in the graded doses (10 mg, 20 mg and 50 mg/kg). CCHC-1 and CCHC-2 in the doses of 20 mg and 50 mg/kg produced significant antinociceptive activity as evident from increase in the reaction time. CCHC-3, though increased the reaction time, it was not statistically significant. Morphine (10 mg/kg, i.p.) showed significant ($P < 0.001$) analgesia (Table II).

(2) By NaCl induced writhing test: Intraperitoneal administration of CCHCs in the doses of 10 mg, 20 mg and 50 mg/kg produced protection against the writhing response as following (Table III).

CCHC-1: It showed protection in 60-90% animals.

CCHC-2: In the doses of 20 mg and 50 mg/kg, it produced protection in 70-80% animals.

CCHC-3: It did not show protection against the writhing response to any significant extent

Morphine: Morphine (10 mg/kg) exhibited protection in 100% animals.

Aspirin: It showed protection in 70% animals.

DISCUSSION

In both tail flick technique and NaCl induced writhing method, CCHCs produced

TABLE III : Effect of CCHCs on NaCl induced writhing in rats.

	Group (n-10)	*Dose (ml+, mg/kg,ip) oral#*	*% of animals showing absence of writhing*
I	**Control**		
	D.W.	10+	00
	Vehicle	10+	10
II	**Test drugs:**		
	CCHC-1	10	60*
		20	90**
		50	90**
	CCHC-2	10	20
		20	70*
		50	80**
	CCHC-3	10	20
		20	20
		50	30
III	**Standard drugs:**		
	Morphine	10	100***
	Aspirin	50#	70**

D.W. = Distilled Water *P < 0.05 **P < 0.01 ***P < 0.001
n = Number of animals

significant antinociception. Various heterocyclic derivatives have been found to produce analgesic effect (9, 10, 11). This support our findings as hemicyaninocolourants too are heterocyclic compounds. If we compare the activity of different CCHCs in our study taking into consideration of different groups in the molecules, CCHC –1 was more active perhaps due to chloro-substitution in X position and nitro group in Y-position of molecule. In piperidine and phenyl piperidine analgesics e.g. loperamide, chloro-substitution makes the compound more effective (12).

In view of analgesic activity of CCHCs, it can be concluded that many more preclinical and clinical experiments will be required before establishing their therapeutic efficacy in human beings as well as to understand the mechanism of action.

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