The anaesthetic potency of benzocaine-hydrochloride in three freshwater fish species

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Anaesthesia was induced in the common carp, *Cyprinus carpio*, tilapia, *Oreochromis mossambicus* and rainbow trout, *Salmo gairdneri*, at concentrations of 25; 50; 75 and 100 mg/l of benzocaine-hydrochloride as well as neutralized benzocaine-hydrochloride at water temperatures of 15; 20 and 25 °C. The results obtained indicated intra- and interspecific differences in the susceptibility of fish to anaesthesia due to metabolic, chemoreceptive and temperature tolerance differences in and amongst the three species.

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Bensokaïenhidrochloried en geneutraliseerde bensokaïen-hidrochloried is gebruik in konsentrasies van 25; 50; 75 en 100 mg/l water om narkose te induseer by die gewone karp, *Cyprinus carpio*, die reënboogforel, *Salmo gairdneri* en die bloukurper, *Oreochromis mossambicus*. Die eksperiment is uitgevoer by drie verskillende watertemperature te wete 15; 20 en 25 °C met visse wat vooraf by hierdie onderskeie temperature geakklimatiseer is. Die resultate wat behaal is dui op definitiewe intra- sowel as interspesie verskille in die visse se vatbaarheid vir narkose. Hierdie verskille skyn die gevolg te wees van verskille in metabolisme, chemoresepsie en temperatuurbestandheid binne 'n betrokke spesie sowel as tussen die drie spesies.

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Tricaine methane sulphonate (MS 222-SANDOZ) is commonly used as an anaesthetic in fish research (Smit 1980), but it is relatively expensive. We attempted to find an equally effective, but less expensive alternative anaesthetic. A water soluble benzocaine-hydrochloride compound (BH), a benzoic acid derivate which shows structural similarities to MS 222, was synthesized (Ferreira, Smit, Schoonbee & Holzapfel 1979a). The suitability of this substance in fish physiological research was assessed and it compared very favourably with MS 222 in inducing anaesthesia in freshwater fishes (Ferreira *et al.* 1979a). We have now compared the anaesthetic potency of four different concentrations of BH on three freshwater fish species at three different water temperatures. According to Smit & Hattingh (1979) neutralized MS 222 induces a deeper, more consistent anaesthesia in fish than MS 222 itself and because of the structural similarities between MS 222 and BH, it was likewise decided to use neutralized BH and to compare its potency with that of BH.

Materials and Methods

Healthy specimens of both sexes of the common carp, *Cyprinus carpio* and the rainbow trout, *Salmo gairdneri*, were obtained from the Fisheries Research Stations at Marble Hall and Lydenburg, Transvaal, South Africa, respectively. Specimens of the freshwater bream, *Oreochromis mossambicus* were obtained from the Hartbeespoortdam Provincial Fish Hatcheries. All the fish were acclimatized for at least two months in 1 000-ℓ aquaria after which they were kept at temperatures of 15; 20 and 25 °C for three weeks. Prior to the investigation, each individual fish was kept separately in a 50-ℓ aquarium for 24 h to minimize possible effects of stress. The mass and the age of the fish used were known.

Anaesthetization was carried out with BH and neutralized BH (NBH) at concentrations of 25; 50; 75 and 100 mg/l of aquarium water and the experiments were conducted at water temperatures of 15; 20 and 25 °C with fish acclimatized at these temperatures. For each concentration of anaesthetic employed 10 fish of each species were used. Anaesthetic induction times were recorded from the time of addition of the anaesthetic to the aquaruim water until the fish in it turned on its side without being able to correct its balance (stage 3 of anaesthesia; McFarland 1959). Thereafter, each fish was immediately removed and weighed before being transferred to another tank with clean water and similar environmental conditions. The time recorded from removal of the fish from the aquarium containing the anaesthetic until the righting reflex was observed was taken as the recovery time from anaesthesia. The rates

Table 1 Anaesthetic potency of benzocaine-hydrochloride (BH) and neutralized benzocaine-hydrochloride (NBH) in *Oreochromis mossambicus* at three different temperatures (n = 10)

	Concentration of anaesthetic (mg/l)									
	0		25		50		75		100	
	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
Temperature: 15 °C										
BH										
Induction time (min)	–	–	15,00	0,00	9,62	0,79	7,67	0,72	7,39	0,32
Recovery time (min)	–	–	0,00	0,00	1,82	0,22	2,06	0,37	2,44	0,72
Mouth movements (per min)	40,00	2,00	27,00	2,00	22,50	2,84	16,67	1,75	17,33	1,75
Opercular movements (per min)	42,00	2,00	29,00	2,00	25,33	2,16	17,67	1,75	18,33	2,07
NBH										
Induction time (min)	–	–	15,00	0,00	9,53	0,96	6,97	0,44	4,15	0,31
Recovery time (min)	–	–	–	–	0,85	0,21	1,99	0,56	2,88	0,32
Mouth movements (per min)	40,00	2,00	32,00	2,05	29,00	1,01	27,33	1,71	24,08	1,74
Opercular movements (per min)	42,00	2,00	34,25	1,26	29,33	1,94	28,33	1,71	25,67	2,12
Temperature: 20 °C										
BH										
Induction time (min)	–	–	15,00	0,00	8,79	0,81	5,41	0,54	2,45	0,54
Recovery time (min)	–	–	0,00	0,00	1,35	0,22	1,57	0,29	2,21	0,35
Mouth movements (per min)	51,29	1,41	37,00	0,71	29,25	1,94	24,08	2,01	24,00	2,91
Opercular movements (per min)	52,61	1,31	38,83	0,68	31,25	1,96	25,08	2,01	25,00	2,91
NBH										
Induction time (min)	–	–	15,00	0,00	8,10	0,73	4,20	0,51	1,74	0,20
Recovery time (min)	–	–	–	–	0,80	0,15	1,98	0,45	2,34	0,33
Mouth movements (per min)	51,29	1,41	44,81	44,81	41,51	0,49	38,45	0,75	35,24	1,67
Opercular movements (per min)	52,61	1,31	46,21	46,21	42,77	0,68	39,45	0,75	37,09	1,81
Temperature: 25 °C										
BH										
Induction time (min)	–	–	15,00	0,00	10,32	0,88	5,79	0,64	3,23	0,37
Recovery time (min)	–	–	0,00	0,00	0,85	0,19	1,43	0,43	1,99	0,43
Mouth movements (per min)	60,00	1,87	47,00	2,02	43,25	3,06	33,83	3,88	33,00	2,54
Opercular movements (per min)	62,00	1,87	52,00	2,12	44,25	2,06	38,71	3,66	36,67	1,66
NBH										
Induction time (min)	–	–	15,00	0,00	9,90	0,94	4,71	0,59	1,78	0,31
Recovery time (min)	–	–	–	–	00,50	0,26	1,60	0,43	2,05	0,28
Mouth movements (per min)	60,00	1,87	55,21	1,36	51,67	1,62	48,27	1,38	45,42	1,54
Opercular movements (per min)	62,00	1,87	56,49	1,71	52,84	1,73	49,27	1,38	46,39	1,32

of mouth and opercular movements of each fish were recorded before the addition of the anaesthetic to water and then at half-minute intervals until stage 3 of anaesthesia had been reached.

Neutralized BH was prepared by dissolving the required amount for each aquarium in 100 ml aquarium water and adding to that a 100 ml equimolar solution of NaOH also made up in aquaruim water.

Results

Increasing concentrations of BH resulted in shorter anaesthetic induction times for all three species investigated (Tables 1, 2 & 3) (r = −0,93 for *O. mossambicus*; r = −0,94 for *C. carpio* and r = −0,93 for *S. gairdneri*) and were even further reduced with the use of NBH (r = −0,96 for *O. mossambicus*; r = −0,95 for *C. carpio* and r = −0,95 for *S. gairdneri*).

As far as temperature is concerned it was noted that for the same concentration of BH and NBH employed, the anaesthetic induction times decreased with an increase in temperature for *S. gairdneri* (Table 2). However, induction times for *C. carpio* (Table 3) and *O. mossambicus* (Table 1) showed a decrease between 15 °C and 20 °C but a slight increase at 25 °C compared with the results achieved at 20 °C.

Anaesthetic recovery times also increased with increasing concentrations of BH and NBH with NBH producing longer recovery times than BH for all three species. An increase in temperature resulted in shorter recovery times for *O. mossambicus* and *C. carpio* while the shortest recovery times in the case of *S. gairdneri* were recorded at 20 °C and the longest recovery times for this species at 15 °C. At 25 °C the recovery times for *S. gairdneri* were slightly longer than at 20 °C.

No correlation was found between the mass of individual fish and the concentration of the anaesthetic. This was also the case with anaesthetic induction and recovery times. The age of the fish also showed no correlation with these above-mentioned parameters.

The mean values for the rate of the mouth and opercular movements in all three fish species during anaesthesia induc-

Table 2 Anaesthetic potency of benzocaine-hydrochloride (BH) and neutralized benzocaine-hydrochloride (NBH) in *Salmo gairdneri* at three different temperatures (n = 10)

	Concentration of anaesthetic (mg/l)									
	0		25		50		75		100	
	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
Temperature: 15 °C										
BH										
Induction time (min)	–	–	7,90	0,48	2,89	0,36	1,71	0,25	0,82	0,08
Recovery time (min)	–	–	1,07	0,49	1,24	0,27	1,74	0,26	2,34	0,18
Mouth movements (per min)	75,00	1,30	61,00	1,41	56,08	2,83	52,28	3,40	46,83	3,54
Opercular movements (per min)	75,00	1,30	63,00	1,41	59,25	2,60	54,46	3,58	48,83	3,54
NBH										
Induction time (min)	–	–	7,60	0,83	2,75	0,85	1,67	0,31	0,80	0,10
Recovery time (min)	–	–	1,27	1,60	1,61	2,00	1,83	2,20	2,39	0,63
Mouth movements (per min)	75,00	1,30	68,08	1,75	65,11	1,49	63,29	2,01	61,47	1,27
Opercular movements (per min)	75,00	1,30	70,21	1,64	66,28	1,53	64,19	1,79	61,47	1,27
Temperature: 20 °C										
BH										
Induction time (min)	–	–	7,14	0,32	1,69	0,30	1,18	0,20	0,79	0,09
Recovery time (min)	–	–	0,87	0,39	1,19	0,33	1,47	0,31	2,10	0,40
Mouth movements (per min)	98,00	1,75	85,00	1,30	81,17	2,79	76,69	3,27	71,83	2,79
Opercular movements (per min)	98,00	1,75	85,00	1,30	81,17	2,79	76,67	3,27	71,83	2,79
NBH										
Induction time (min)	–	–	6,75	0,75	1,68	0,21	1,05	0,18	0,68	0,07
Recovery time (min)	–	–	0,63	0,21	1,38	0,36	1,56	0,53	2,03	0,49
Mouth movements (per min)	98,00	1,75	82,14	1,16	83,18	1,09	79,26	1,47	77,11	1,63
Opercular movements (per min)	98,00	1,75	89,26	1,21	83,89	1,59	79,82	1,38	78,42	1,49
Temperature: 25 °C										
BH										
Induction time (min)	–	–	2,47	0,14	1,73	0,14	0,74	0,09	0,70	0,10
Recovery time (min)	–	–	0,96	0,24	1,77	0,19	2,65	0,20	2,74	0,27
Mouth movements (per min)	110,00	2,45	99,00	0,63	95,08	2,01	90,67	1,86	87,83	3,33
Opercular movements (per min)	110,00	2,45	99,00	0,63	95,08	2,01	90,67	1,86	87,83	3,33
NBH										
Induction time (min)	–	–	2,22	0,73	1,38	0,34	0,63	0,13	0,60	0,14
Recovery time (min)	–	–	0,98	0,36	1,27	0,22	2,15	0,51	2,38	0,78
Mouth movements (per min)	110,00	2,45	91,26	2,01	87,62	1,64	81,00	1,82	79,00	2,01
Opercular movements (per min)	110,00	2,45	92,31	1,77	88,75	1,39	82,44	1,47	80,80	2,01

tion decreased with an increase in the concentration of BH and NBH employed. This is associated with a decrease in the induction times in all three species.

A rise in temperature resulted in increasing mouth and opercular movements in the three species for each of the concentrations of anaesthehtic used (Tables 1, 2 & 3). In all cases the mean values obtained for mouth and opercular movements, during the induction of anaesthesia, were generally lower than the initial values recorded for unanaesthetized fish. *Oreochromis mossambicus* was found to have the lowest rate of mouth and opercular movements for all concentrations of BH and NBH employed and all three temperatures. In the case of *Cyprinus carpio* values were higher in all cases, while *Salmo gairdneri* showed the highest mouth and opercular rates for all three temperatures and all concentrations of the anaesthetics employed.

Discussion

The pronounced intra- and interspecific differences in the anaesthetic potency of benzocaine-hydrochloride obtained at the three temperatures may be the result of morphologieal, metabolic, osmoregulatory and chemoreceptive differences amongst these three species.

The natural environment in which these fish live as well as their ability to cope with changes in environmental conditions may also influence their susceptibility to anaesthesia. *S. gairdneri* is a coldwater fish whilst *O. mossambicus* is a warmwater fish. *C. carpio* has the ability to adapt to a wide range of temperature and environmental conditions (Smit, Hattingh & Ferreira 1981). Marking (1967) and Houston & Woods (1976) also showed that the anaesthetic potency of a drug depends on the temperature of the water at which anaesthesia is induced. The results of this study confirm these findings because the induction of anaesthesia at different water temperatures resulted in different anaesthetic induction times for all three species.

An increase in water temperature resulted in shorter anaesthetic induction times in the case of *S. gairdneri* and accor-

Table 3 Anaesthetic potency of benzocaine-hydrochloride (BH) and neutralized benzocaine-hydrochloride (NBH) in *Cyprinus carpio* at three different temperatures (n = 10)

	Concentration of anaesthetic (mg/l)									
	0		25		50		75		100	
	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
Temperature: 15 °C										
BH										
Induction time (min)	–	–	15,00	0,00	5,27	0,48	2,35	0,20	1,68	0,17
Recovery time (min)	–	–	0,00	0,00	2,56	0,34	2,80	0,23	3,62	0,30
Mouth movements (per min)	55,00	2,09	46,00	1,41	40,67	2,73	34,67	2,34	30,33	3,45
Opercular movements (per min)	58,00	8,09	49,00	1,41	41,67	2,73	35,67	2,34	31,33	3,46
NBH										
Induction time (min)	–	–	15,00	0,00	5,10	0,68	1,91	0,41	1,22	0,11
Recovery time (min)	–	–	–	–	2,00	0,53	2,73	0,31	3,61	0,37
Mouth movements (per min)	55,00	2,09	52,43	1,42	49,38	1,44	44,00	1,21	41,61	1,34
Opercular movements (per min)	58,00	8,09	54,28	1,57	50,26	1,36	45,00	1,21	42,47	1,39
Temperature: 20 °C										
BH										
Induction time (min)	–	–	15,00	0,00	4,70	0,42	1,74	0,17	1,01	0,11
Recovery time (min)	–	–	0,00	0,00	2,00	0,45	2,33	0,22	2,89	0,37
Mouth movements (per min)	75,88	1,58	68,83	1,94	64,67	3,44	59,00	4,15	52,00	3,74
Opercular movements (per min)	76,88	1,58	70,88	1,94	68,17	3,31	62,83	3,87	57,17	4,02
NBH										
Induction time (min)	–	–	15,00	0,00	3,76	0,54	1,60	0,19	0,88	0,12
Recovery time (min)	–	–	–	–	1,30	0,28	1,85	0,26	3,06	0,51
Mouth movements (per min)	75,88	1,58	75,27	1,73	69,12	1,14	65,00	1,28	63,29	1,52
Opercular movements (per min)	76,88	1,58	73,41	1,64	69,77	1,02	66,00	1,28	64,37	1,32
Temperature: 25 °C										
BH										
Induction time (min)	–	–	15,00	0,00	5,48	2,39	2,01	0,24	1,05	0,14
Recovery time (min)	–	–	0,00	0,00	1,54	1,74	2,13	0,34	2,43	0,21
Mouth movements (per min)	95,44	1,41	88,00	3,85	86,33	3,56	81,83	3,76	79,62	5,39
Opercular movements (per min)	97,00	1,09	89,33	8,16	90,33	3,56	85,33	4,32	78,83	3,55
NBH										
Induction time (min)	–	–	15,00	0,00	5,66	0,71	1,97	0,19	0,97	0,11
Recovery time (min)	–	–	–	–	1,29	0,25	2,03	0,18	2,37	0,36
Mouth movements (per min)	95,44	1,41	91,00	1,07	89,31	1,71	87,28	1,29	85,00	1,41
Opercular movements (per min)	97,00	1,09	93,00	1,07	90,28	1,62	88,36	1,37	86,00	1,41

ding to Clark (1948) and Bullock (1955) this may be the result of higher metabolic activity at higher temperatures resulting in a rapid uptake of the drug. Smit *et al.* (1981) suggested that *S. gairdneri*, a coldwater fish, may experience a collapse of osmoregulatory and other functions at high temperatures. Results obtained from *O. mossambicus* and *C. carpio* showed a decrease in anaesthetic induction times with a rise in temperature between 15 and 20 °C. This is probably due to higher metabolic activities as suggested earlier. A further rise in the water temperature to 25 °C resulted in an increase in anaesthetic induction times. This again may point to the fact that both these species have a preference for warmer waters and therefore may be more able to withstand the effects of the anaesthetic at the higher temperatures.

The higher metabolic activity with an increase in temperature is the result of a decrease in dissolved oxygen in the water coupled with a rise in temperature. This means that an increase in respiratory activities is required for the maintenance of sufficient oxygen levels in the fish (Schmidt-Nielson 1975). The increase of respiratory activities with a rise in temperature was clearly demonstrated in this study by an increase in the rate of mouth and opercular movements in all three species with an increase in temperature.

The decrease in induction times with an increase in the concentration of BH and NBH is to be expected because of the higher concentration gradient which favours a more rapid uptake of the anaesthetic. The influence of mass and age of individual fish on the anaesthetic potency of BH was also investigated. No correlation, however, was found between these factors and the rate of induction of anaesthesia, which results agree with the findings of McFarland (1959).

The physiological reactions of the fish to the anaesthetic in the water as well as the effect of the anaesthetic on the water itself, are also important in the evaluation of the anaesthetic potency of the substance. Ferreira *et al.* (1979b) and Smit (1980) showed that anaesthetics can induce certain changes in the water chemistry which may influence the eventual uptake of the anaesthetic by the fish. After addition of the anaesthetics

O. mossambicus immediately reacted by clamping its jaws for variable lengths of time resulting in low mean rates of mouth and opercular movements which in turn seemed to allow for a slower uptake of the drug. This is substantiated by the fact that *O. mossambicus* required the longest induction times in all cases. This reaction, however, seldom occurred with *C. carpio* and *S. gairdneri*. With the use of NBH this irritated response did not appear. The use of NBH thus resulted in higher mean rates of mouth and opercular movements in all three species and therefore shorter induction times than found with BH as a result of a more rapid uptake of this un-ionized substance (Hunn & Allen 1974; Ferreira 1982). Recovery time after the induction of anaesthesia was also affected by the same factors that influenced induction times. An increase in the concentration of BH resulted not only in longer recovery times but also in longer induction times for a specific concentration of the anaesthetic. Recovery of the fish was generally quicker at high water temperatures than at low temperatures because of the faster metabolic rate of the fish at higher temperatures (Schmidt-Nielson 1975). The exception, however, was *S. gairdneri* at 25 °C which took fairly long to recover. This again supports the suggestion that this coldwater fish suffers a collapse in osmoregulatory and metabolic functions at this relatively high temperature.

It should thus be obvious that the anaesthetic potency of benzocaine-hydrochloride may be influenced by several factors which must at all times be borne in mind if safe and effective anaesthesia is to be ensured.

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