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SCREENING OF CLONIDINE EFFECT ON BRAIN ACETYLCHOLINE CONTENT, ANTICON VULSANT AND LOCAL ANAESTHETIC PROPERTIES

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ABSTRACT

Clonidine, a potent centrally acting antihypertensive drug was screened for effect on “free” and “bound” acetylcholine (Ach) in brain of morphine dependent and morphine withdrawal rats, anticonvulsant activity and morphine withdrawal rats, antconvulsant activity and local anaesthetic activity. “Free” and “total” Ach content in normal (nondependent) animals as well as in animals dependent to morphine was not changed by eonidine. Naloxone produced abstinence syndrome in dependant rats with highly significant increase in “free” Ach. Clonidine pretreatment decreased the rise of “free” Ach content produced by naloxone. It failed to show any anticonvulsant effect against maximal electroshock convulsions in rats, however, it exhibited significant local anaesthetic activity. Diuresis, piloerection and convulsions were observed with higher doses of clonidine.

Introduction

Clonidine, a centrally acting antihypertensive alpha-2 adrenoceptor agonist, has been shown to alleviate morphine abstinance syndrome in man and animals, (Agha Janian, et al 1973; Gold, M. Sctal 1978). It has also been shown to possess many other diverse effects, on tissues, analgesic effect, anticonvalsant property, inhibition of conditioned avoidance response etc. In brain, Ach is present in “free” and “bound” forms. The former is active and available for release and influences “total” Ach content, meaning thereby that increase or decrease in "free" Ach content leads to increase or decrease of “total” Ach content (Crossland, J. 1973). Further, adrenergic drugs have been shown to influence Ach release (Deck, R. et al 1971; Kroneberg, G. et al, 1967). Keeping these facts in Hew, the present work was undertaken to investigate effect of clonidine on “free” and “bound” Ach in the brain of rats where experimentally morphine withdrawal was produced. In addition to effect on brain Ach, anticonvalsant and local anaesthetic properties of clonidine were also investigated.

Materials and Methods

Animals used in the present study were Wister albino rats, albino rabbits and guinea-pigs of either sexes. Morphine and naloxone were employed for producing dependance and withdrawal respectively. Lidocaine was used as a standard drug for comparing local anaesthetic effect of clonidine.

Morphine dependence was induced in tats by feeding them morphine in 45% sucrose solution for four weeks. Initially, concentration of morphine was 0.1 percent which war increased by 0.1 percent every weak leading to 0.4 percent in the 4th week (Hunt, W.B. 1971).

For withdrawal syndrome, naloxone was administered intraperitoneally in a dose of 4 mg/kg. Withdrawal symptoms were scored as per method described by (Hunt, W.B. 1971).

Brain Ach Content:

The rats (150g to 200g body weight) were divided in five groups, each group consisting of five animals. Morphine dependence, and drug treatment were instituted in rats of different groups as shown in Table 1.

Animals of group 1 to IV *were* made morphine dependent while group V rats were non-morphine dependent animals to whom only sucrose solution was given for 4 weeks. Saline (0.9 percent), clonidine and naloxone were administered intraperitoneally on the day of Ach extraction.

Table 1: Showing design of drug(s) Administration in different Groups before Extraction of "Free" and "Bound" Acetylcholine from Brain

Group (5 rats in each)	Drug Treatment		Morphine Dependence
	1st administration.	2nd administration after 15 min. of 1st administration.	
I	Saline	Saline	Instituted
II	Saline	Clonidine	Instituted
III	Clonidine	Naloxone	Instituted
IV	Saline	Naloxone	Instituted
V	Saline	Saline	Not Instituted

After 1 min of second administration, animal was sacrificed, brain homogenized and "free" and "total" Ach were extracted according to method described by Crossland and Slater (Crossland, J et al, 1968). It was then bio-assayed on frog's rectus abdominis muscle preparation using eserinized (1×10^{-5} g(ml) Ringer solution (Crossland, J. 1953).

Anticonvulsant activity:

Anticonvalsant property of clonidine was evaluated in albino rats of either sex weighing between I50g and 200g by producing maximum electroshock seizures, (Hendley, C. D et al 1948). Test drug (clonidine) was administered in different doses starting from 0.01 mg/kg, increasing in logerathmic ratio to different groups of animals intraperitoneally 30 min prior to electroshock. Prevention of tonic extensor space was kept as criteria ^{of} protection.

Local anaesthetic activity:

It was determined by the following methods using lidocaine hydrochloride as a standard for comparison.

Surface anaesthesia:

Albino rabbits of either sex (1.5-2.0 kg) were used. 0.25 ml of 0.1 and 1.0% solutions of clonidine or lidocaine respectively, was instilled in the conjunctival sac of one eye and isotonic saline was applied to other eye. The cornea was touched with a hairaesthesiometer 6 times in succession at intervals of 5 min (out of 36) the blink reflex failed to occur in the drug treated eye during the 30 min period was added up, and using this sum minus the sum of stimuli which failed to elicit blink reflex in contralateral control eye treated with saline indicated the degree of anaesthesia (Murmann, W et al, 1966). 6 observations were made for each concentration of different drugs.

Infiltration anaesthesia:

Guinea-pigs of both sexes weighing between 300 and 500g were used. Two areas of 3 x 60 cm on the back were shaved. 0.25 ml solution of clonidine (0.1) or lidocaine (l%) and isotonic saline were injected intracutaneosly in the areas and the wheals formed were outlined with ink. The test for anaesthesia consisted in application of a prick with a pin. After observing the normal response of the guineapig to the prick applied outside the wheal, that is, contraction of the surrounding skin, 6 pricks were applied at intervals of 5 sec inside the wheal. The procedure was repeated ev*ery* 5 min for 30 min. The number of negative responses out of 36 in drug-induced wheal were added up, this sum minus the sum of negative responses during 30 min in the wheal caused by isotonic saline gave the degree of anaesthesia (Bulbring, E. et al 1945).

Results

No significant change was observed in Ach content of brain of morphine dependant (group 1) and non-morphine dependent (group V) rats.

Naloxone administration produced withdrawal syndrome which had grading of 5.2. Brain Ach content was increased significantly (group I & IV).

Clonidine, when administered in morphine dependent rats, did not change brain Ach content. The results are not significant statistically (group I and II). When clonidine was administered before naloxone (group III), a mild abstinance syndrome of less than 1 grade developed. Brain Ach content though insignificantly was more in clonidine – naloxone treated (group III) animals than saline – saline treated (group I) animals, but was significantly less than brain Ach content of saline – naloxone treated (group IV) animals (Table II).

Anticonvulsant effect:

Clonidine in doses of 0.01, 0.03 and 0.1 mg/kg. failed to protect animals a against maximum electroshock seizures. Dose of clonidine was not exceeded beyond 0.1 mg/kg because animals showed mild convulsions with larger doses. All the animals (up to 0.1 mg/kg dose of clonidine) showed phoerection, fine tremors and increase in urinary out put.

Table 2: "Free", "bound" and "Total" Acetylcholine Content of Brain and Score of Morphine withdrawal Symptoms

Group	Acetylcholine content of brain Scg/g ± SE			Free/Total acetylcholine content percent ± SE	Score of withdrawal symptoms
I	0.32±0.03 (a)	2.20 ± 0.06 (a)	2.52±008 (a)	12.69±160 (a)	–
II	0.34±0.01 (a)	2.12±007 (a)	346±006 (a)	12.86±1.60 (a)	–
III	044±0.05 (a)	2.00±0.05 (a)	244±006 (a)	18.09±1.87 (a)	<1
IV	0.89±0.03 (c)	2.03±0.03 (b)	291±005 (h)	30.58±0.80 (c)	5.2
V	0.31±0.24	2.37±0.24	268±0.25	1196±080	–

*Figures are mean of 5 observations in each group.
*P value (in parenthesis): (a) >0.05, (b) <0.05, (c) <0.00

Table 3: Local Anaesthetic Activity of clonidine and Lidocaine by Guinea Pig Heal Method

Drug (% Solution)	Number of Observations	Degree of Anaesthesia ± SE*	Potency ** (%)
Cloridine (0.1)	6	290±008	80.5
Lidocaine	6	344±098	94.4

*Degree of Anaesthesia: Mean ± SE
**Potency is represented as % of mean failure of response out of 36 stiulations.

Local anaesthetic effect:

Surface anaesthesia: On local instillation of 0.01, 0.03 and 0.1 solution of clonidine in the *eyes* of rabbit, no surface anaesthetic effect was observed. All the animals developed conjunctival congestion, blapherespasm and photopholia indicating local irritation.

Infilltratiun anaeathesia:

On intradermal administration of clonidine, in concentration of 0.1% in guinea-pig, local annaesthetic effect produced by 1.0% solution of lidocaine. The results are summarized in Table III.

Discussion

In the present study clonidine has significantly decreased abstinence syndrome produced by administration of naloxone in morphine dependent rats which is in accordance with recently observed clinical study of the beneficial effect on the course of morphine abstinance syndrome human volunteers. The rise of 'free' Ach content has shown to have a direct correlation with intensity of morphine withdrawal syndrome (Cross-land J. et al 1979). In the present study, a significant decrease has been observed in "tree" Ach content when morphine dependent animals were given clonidine prior to naloxone in production of withdrawal syndrome. Further, Ach has been shown to he one of the transmitter substance involved in morphine analgesia and abstinance syndrome (Dole, V.P. 1976). It can be deduced that rise in "free" Ach in abstinance syn. time may be due to an increase in its release from cortex, which is supported by the observations of others (Crossland, J. 1973).

Decrease in rise of Ach content by clonidine in morphine withdrawal indicates strongly the presence of presynaptic adrenoceptors on contral cholinergic neurones, stimulation of which leads to an inhibition of Ach release. The Hypothesis is supported indirectly by the observation that clonidine inhibited contraction of the electrically stimulated guinea-pig ileum in vitro" which is pretreated with phentolamine, an alpha-adrenergic blocking agent (Deck, R. et al 1971). Previously, workers have shown that this action of clonidine was due to inhibition of Ach release from postganglionic parasym-pathetic elements in the ileum (Deck. R. et al, 1971. Kroneberg, G. et al. 1967), it has been proved *that* a similarity exists between isolated guinea-pig ileum and mammalian Brain (Deck, R. et al. 1971).

Clonidine treatment has not changed “free” and “bound” Ach content of normal (morphine non-dependent) as well as morphine dependent animals. To any significant level in contrast to its significant effect when abstinance syndrome was instituted by naloxone. It may be explained due to too low release of transmitter which remains uneffected by the dose of clonidine used in the present study. Neverthless, for a more reasonable and aunthentic explanation more elucidation is warranted.

Clonidine has failed to protect rats from maximum electroshock convulsions which is contrary to the observations of (Kulkarni, 1981). All the animals receiving clonidine showed tremors in lower doses and clonic convulsions in dose of 1.0 mg/kg which pre-vented us from investigating its ability to protect against chemoshock. The cause of this diversity needs further exploration on the lines to elucidate for existence or non – existence of coordination between the change in "free" and "bound" Ach content of brain and tremorogenic/convulsing effect of clot-Milne. Diuresis and piloerection was observed in all the animals receiving clonidine which is in accordance with observations of other workers (Barr, J. G et al, 1979). The piloerection might be due to hypothermia as has been reported in one of the previous study (Tsougaris-Kupfer, D et al 1972).

In lower concentrations clonidine failed to produce surface anaesthesia. Higher concentrations were not tried because of irritant nature of drug. In guinea-pig model, clonidine showed significant local anaesthetic effect in a concentration of 0.1% which was comparable with 1.0% lidocaine,

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